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FORMULATION AND EVALUATION OF HYDROGEL BASED TOPICAL DELIVERY OF BAEL (Aegle marmelos) FRUIT EXTRACT FOR WOUND HEALING.

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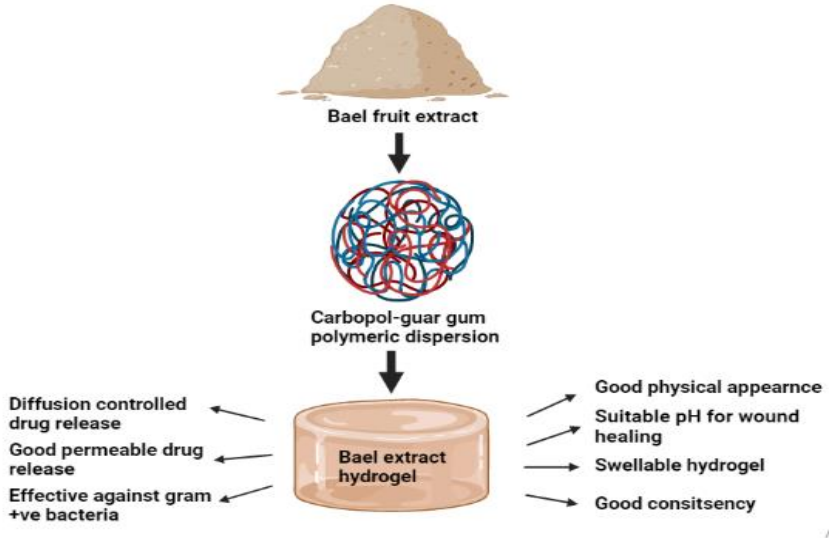
ABSTRACT

Wounds are physical injuries that cause the skin to open or break, or a break in the epithelial integrity of the skin, as well as disturbance of the structure and function of underlying normal tissue. Medicinal plants play indispensable roles to treat various ailments. In the current research bael fruit extract hydrogel was prepared and optimized with the aim to reach effective concentration at wound site and make it an effective drug delivery system in the wound care. The hydrogel was developed by co-polymeric dispersion method. The prepared hydrogel of different polymeric ratio were evaluated for various parameters. The ideal formulation batch was selected for *ex vivo* skin permeation studies using *Albino Wister* rat skin. The formulated hydrogel was found to be brownish translucent in nature. The solubility of extract was 850, 80.18 and 70.75 mg/ml in water, methanol and hydro alcoholic solvent respectively. 3.296 was the partition coefficient of the extract determined using n-octanol-water. FTIR studies revealed that there was no possible interaction between the extract and the polymer. The drug content of the hydrogel ranged from 89.66% - 90.32%**.** The viscosity of the formulation was found to be in the range of 7573 to 18342 cps. The pH was found to be varying between 3.362 and 5.4, which was suitable for the application of skin. The swelling ratio was maximum for 60 min. The *ex vivo* permeation of extract from the hydrogel (F6) was found to be 72.64 % at the end of 24 hrs. Release kinetics of formulated hydrogel exhibited non fickian /anomalous diffusion. F6 formulation significantly increased wound contraction rate compared to the negative control group and control group ($P < 0.05$). From the results obtained it can be concluded that, bael fruit extract based hydrogel can be considered for the attenuation of wound healing in gram positive bacteria due to its free radical scavenging and anti-oxidant activity.

Key words: *Aegle marmelos*, hydrogel, release kinetics, wound healing, anti-oxidant activity, free radical scavenging activity.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Herbal products have become more popular in industrialized nations in recent decades. The safety of herbal products has become a key concern in public health due to their popularity and global commercial development.

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The hunt for physiologically active chemicals in plant extracts has often captivated researchers' curiosity in discovering novel medication sources.[1] Natural vegetation and herbal remedies are quite popular in India.[2] The primary benefit stated for medicinal plant therapeutic applications in various diseases are their safety, as well as their affordability, effectiveness, and ease of access.[3]

India's most fabulous ancient medicine is *Aegle marmelos,* which has been proved potential drug.[2] Many bioactive chemicals have been identified from this plant, which has significant traditional benefit against different illness.[4] *Aegle marmelos* Linn. is a perennial, belonging to family Rutaceae, commonly known as Bael. It has a mucilaginous flavour and a mild aromatic odor. This tree's root, leaf, trunk, fruit, and seed are all beneficial for a variety of illness. Ayurvedic practitioners utilize virtually all of their components, but the fruits have the highest therapeutic potential.[2, 5, 6]

Bael contains a wide range of phytochemicals, including alkaloids, tannins, essential oils, gums, resins, coumarin, and polysaccharide, making it useful against a wide range of illness. [7]

The current study was started to create and assess *Aegle marmelos* hydrogel based topical administration for wound healing based on its use in traditional practices. Wounds have become one of the leading common causes of death across the world. There is an immediate requirement for a wound dressing that protects the wound bed while also encouraging skin regeneration to speed up wound healing.[8] Hemostasis, inflammation, proliferation (cellular infiltration, angiogenesis, and re-epithelialization), and maturation/remodelling are the four stages of wound repair, which occur in a time sequence but overlap.[9]

Aegle marmelos' antioxidant action might be attributed to the inactivation of free radicals, the formation of complexes with metal ions, or a combination of the two[10] . Both extracts included steroids, terpenoids, flavonoids, phenolic compounds, lignin, fat and oil, inulin, proteins, and carbohydrates. Saponins and cardiac glycosides are only found in aqueous extracts, whereas alkaloids are only found in alcoholic extracts. Flavonoids and tannins are a class of chemicals that function as main antioxidants and free radical scavengers.[11,12]

The presence of phytochemicals like flavonoids, hydroxyls and other polyphenolic substances might explain the antioxidative properties. Polyphenols have been shown to have therapeutic and physiological properties. Other phytoconstituents, which include alkaloids, essential oils, and sterols, are responsible for bael's wound healing activity, causing an increase in epithelization, wound contraction, tensile strength, and hydroxyproline content.[13]

Antibacterial hydrogels have recently been created as wound dressings because they can keep the area wet, inhibit bacterial infection, allow oxygen to pass through, and be readily removed without causing secondary harm.[14] Hydrogels are soft materials that are often made up of three-dimensional, insoluble, cross-linked polymer networks that can absorb a considerable quantity of water. Chemical or physical interactions frequently cross-link these polymer networks.[15] When the formulation comes into contact with the skin, it forms a semi-occlusive film over the skin, concentrating the active component in a polymer matrix. Because of their important physical and chemical properties, such as controllable and prolonged drug release in organisms, polymeric gels and hydogels are now widely used in drug delivery systems. As a result, high local concentrations of medical preparations are maintained in the affected tissues for a long time.[16, 17]

2. MATERIALS AND METHODS

Bael fruit pulp extract was obtained from Amines Biotech Pvt Ltd, Gujarat, India as a gift sample. Carbopol-940 and Guar gum were procured form Yarrow chemical products, Mumbai, India. Triethanolamine and methyl paraben were purchased form Hi-media laboratories, Mumbai, India, isopropyl myristate was obtained from Merck Life Science Pvt Ltd, Mumbai, India. All the other chemicals obtained were of analytical grade.

2.1 Determination of solubility

The solubility of the drug was determined in distilled water, methanol and hydro-alcoholic by using standard shake flask method. To make a standard solution, an excess amount of extract was obtained and dissolved in a measured amount of the above solvents separately in a glass beaker. To aid in the attainment of equilibrium with the undissolved drug particles, the solution was agitated intermittently. The concentration was measured spectrophotometrically after a measured quantity of the filtered drug solution was removed after 24 hours and gradually diluted with corresponding solvents.[18]

2.2 Determination of partition co-efficient

Before the experiment, the water and n- Octanol were pre-saturated with each other for 24 hours. To the distilled water (10 ml), known quantity of drug was dissolved. 10 ml of octanol was added to equal volume of drug solution in a separating funnel. The system was shaken intermittently for 24 hours. Finally, the layers of water and n-octanol were separated, centrifuged, and analysed by UV spectroscopy.[19]

2.3 Drug polymer compatibility studies

The drug polymer compatibility study was carried out to determine the interaction between the bael fruit extract, carbopol-940, guar gum used for the preparation of hydrogel. The FT-IR was carried out for the pellets of

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extract, carbopol-940 and guar gum, extract polymer mixture separately at wavelength ranging from 4000cm-1 to 400cm-1.

2.4 Preparation of Bael fruit pulp extract loaded topical hydrogel

Bael fruit pulp extract based topical hydrogel was prepared by cold mechanical method. Six Hydrogel formulations of varying polymeric concentrations were prepared. Separately, polyacrylic acid polymer (carbopol 940) and guar gum polymeric dispersions were made by dissolving the calculated amount of polymer in purified water and stirring continuously for 2 hours at 100 rpm until the polymer soaked in water. To neutralise the carbopol and maintain the hydrogel's pH, Triethanolamine was added with constant stirring. . Followed by addition of the necessary amount of methyl paraben as a preservative and then Both polymeric solutions were kept for 24 hours to allow for full swelling and polymer equilibration. Finally, both the polymeric solutions were mixed with continuous stirring by addition of drug solution and isopropyl myristate till extract was finally dispersed in the hydrogel. [20, 21]. The detailed formula is depicted in table 1.

2.5 Evaluation of bael fruit pulp extract loaded topical hydrogel

2.5.1 Physical appearance

Visual observations were used to assess the formulated hydrogel's physical appearance, homogeneity, and texture. .[22]

2.5.2. Determination of drug content

Bael fruit extract was extracted from 1gm of each hydrogel formulation with 10 ml of phosphate buffer pH 5.5 for 30 minutes to test the drug in hydrogels. A UV-visible spectrophotometer was used to determine the amount of extract (drug) released by measuring the absorbance at 281.5 nm.

2.5.3. pH

Each hydrogel composition was weighed and combined with 25 mL distilled water. The pH of the mixture was determined using a pH analyser (Elico India) that had been calibrated with buffer solutions of 4.0, 6.0, and 9.0 before each usage.[23]

2.5.4. Determination of viscosity

The viscosity of the hydrogel formulation was determined using Brookfield viscometer with a spindle T-F spinning at 50 rpm at 25 ºC. Variable stress from 10 to 50 rpm was used to establish the appropriate batch hydrogel viscosity [24]

2.5.5 Swelling Index

To assess the swelling index of the formulated topical hydrogel, one gram of each hydrogel formulation was soaked in 5 ml phosphate buffer pH 5.5 and left for a defined period before being weighed again. After one hour and three hours, the test was repeated. The swelling index was calculated using the formula below. .[25]

$$\text{Swelling index} = (Ws - Wo/Wo) \times 100$$

Where, W_S- weight of swollen hydrogel

W_0 – weight of hydrogel

2.5.6 Spreadability

The spreadability of the formulations was assessed by measuring the spreading diameter of 1 g of sample between two petri plates (15cm * 90cm). The upper plate was given a standard weight ranging from 10 gm to 100 gm until no spreading was noticed [26].

2.5.7 *In vitro* drug release profile

The prepared hydrogel was evaluated for *in vitro* drug release. Dialysis membrane was used to carryout *in vitro* diffusion study using Franz diffusion cell. The Franz diffusion cell was fitted with a cellophane membrane. Formulation was applied on dialysis membrane through donor compartment. The reservoir compartment was filled with 25 mL of pH 5.4 phosphate buffer. The study was carried out at 37 ± 1°C and at a speed of 100 rpm for 24 h. At 1 hour intervals, samples were withdrawn from the reservoir compartment and their absorbance was evaluated spectrophotometrically at 281.5 nm. Each time the reservoir compartment was replenished with the same quantity of 5.5 pH phosphate buffer.[27]

2.5.8 *Ex vivo* permeation study

The abdominal skin of *Albino Wistar* male rats, weighing 150- 200 g, was shaved using a razor after sacrificing by spinal dislocation. For *ex vivo* permeation studies, skins were allowed to hydrate for 1 h before being mounted on the Franz diffusion cell with the stratum corneum (SC) facing the donor compartment. The receptor compartment was filled with phosphate buffer pH 5.5 and receptor phase was maintained at 37 ± 0.5 ºC. 1 g of the gel was placed on the SC side facing the donor compartment. The whole assembly was fixed on a magnetic stirrer, with continuously stirring at 100 rpm. 0.5 ml of solution was withdrawn at pre- determined time intervals (0.15, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hr) and replaced with an equal quantity of buffer into the receptor compartment to maintain sink condition. The amount of drug permeated was determined spectrophotometrically at 281.5 nm[28]

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2.5.9 Release kinetics

Release kinetic studies was carried out to understand the mechanism behind the release and to figure out the best fit plot with the kinetic model. The data obtained from the *in vitro* release studies were fitted to various kinetic model such as Zero order, First order, Higuchi model and Korsmeyer/Peppas model. [29]

2.5.10 Scanning electron microscopy (SEM) images of hydrogel

To compare the structure integrity of formed hydrogel the SEM images of hydrogel without crosslinking and with crosslinking was determined.

2.5.11 Wound healing activity

Wound healing activity was performed by excision wound model. The study was conducted on male albino rats of 200-250 g which were maintained under standard conditions of temperature and humidity. Animals were sedated with subcutaneous injections of ketamine (1 ml/kg) and diazepam (1 ml/kg) on wounding day. The dorsal fur of the animals was shaved with a shaving machine after wound area preparation with 70% alcohol, and the predicted area of the wound to be produced was delineated on the back of the animals on the dorsal thoracic region 1 cm away from the spinal column on the anaesthetized mouse. Using toothed forceps, a scalpel, and scissors, full thickness circular excision incisions of around 300 mm2 were produced along the markings. The wound was blotted with a cotton swab dipped in normal saline to achieve hemostasis. The wound was completely left open. [30, 31]

The rats were divided into three groups (6 mice per group) randomly and each rat was placed in a separated cage. The treatment was done once daily topically in all the cases. The wounding day was considered as day 0. The formulations were applied topically to the respective groups till the wound was completely healed (for a period of 14 days). The wounds were monitored and the area of wound was measured daily. The % of wound contraction was calculated using following formula [32]

$$\% \text{ of wound contraction} = \frac{wound\ area\ on\ day\ zero - wound\ area\ on\ day\ n}{wound\ area\ on\ day\ zero} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Solubility and partition coefficient of Bael fruit extract

The solubility of bael fruit extract was determined in various solvents like water, methanol and hydro alcoholic solution. It was found that aqueous solubility of the extract was comparatively greater than other two solvents. The partition coefficient (P) of the extract in water and n-octanol phase showed 3.296 and Log P was 0.517 which indicates that it has higher concentration in the lipid phase (i.e., the compound is more lipophilic). The results are as represented in table 2.

3.2 Drug polymer compatibility studies

The drug was subjected for FT-IR studies to know the interaction between the drug and polymer used for preparation of hydrogel. The result revealed that there was no possible interaction between the extract and polymer carbopol-940 and guar gum as there was no appearance of new peak and no disappearance of the existing peak, which shows that the polymer did not alter the performance characteristics of drug, thus revealing the compatibility of the selected drug and the polymer. Figure 2 depicts the FTIR spectra of bael and excipients physical mixture.

3.3 Evaluation of formulated hydrogel

3.3.1. Physical appearance

All the formulation showed uniform homogeneity. The physical appearance of the gel formulation was brown transparent in nature. The results are as given in table 3.

3.3.2. Drug content

Drug content was determined to verify uniform dispersion of drug in the hydrogel. The drug content of the gel formulation was in the range of 89.66% to 90.32%. The loss of drug content is attributed to preparation of drug solution. Result of drug content of prepared hydrogels is represented in table 3.

3.3.3. pH

The pH of all developed formulations was in range of 3.362 to 5.4, which is compatible with pH of skin and signifies no irritation to the skin on application. Result of pH of prepared hydrogels is represented in table 3. Studies have shown that acidic environment helped in better wound healing by controlling wound infection and increased antimicrobial activity.

3.3.4. Viscosity

Viscosity of gel formulations reflects consistency. It was measured using Brookfield viscometer T-F spindle at 50 rpm, 25^0C. The formulation viscosity found to be in the range of 7573 cps to 18342 cps. From the results obtained it was evident that, as the polymer concentration increased, viscosity changes. Increase in the polymer

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concentration resulted increased viscosity. Hence, the appearance, viscosity, and skin feel provided by all gel formulations was considered better for a topical gel. Results of viscosity estimation is showed in table 4.

3.3.5. Swelling index

The swelling capacity is an important characteristic of wound healing formulations especially in exudating wounds. Due to their high fluid holding capacity they can absorb a moderate amount of the wound exudates by swelling which leads to formation of a dry bed of wound which further aids into healing process. The swelling index was observed from 32.57 % to 204.98 % and that the polymer concentration indirectly affected the swelling index. Here the formulation F6 with 1.5:0.5 showed controlled swelling. Results of swelling index estimation is shown in table 4.

3.3.6. Spreadability

The spreadability of all the formulations ranged from 2.7 cm to 5.4 cm when a weight of 10 gms to 100 gms was applied at an increment of 10 grams each time. The results depicts that the formulated hydrogels had good spreadability. Table 5 and figure 3 depicts the results of spreadability.

3.3.7. *In vitro* drug release studies

Results of *in vitro* drug release estimation is showed in table 6 and figure 4. From the obtained result it reflected that formulated hydrogel was suitable for controlled and sustained drug delivery. Drug release of different formulations of hydrogels varied from 31.72% to 79.29% depending upon the drug polymer concentration in the prepared hydrogel for 24 h. It showed that F6 formulation i.e. 1.5:0.5 polymeric ratio showed better result than other polymeric ratio. Hence F6 formulation was selected for *ex vivo* skin permeation study.

Selection of ideal batch

Considering results from all the formulation, F6 was found to be suitable for topical application and it was further evaluated for *ex vivo* skin permeation study, release kinetics study, antimicrobial studies and animal studies.

3.3.8. *Ex-Vivo* skin permeation study.

Table 7 and figure 5 depicts the *ex vivo* skin permeation profile of bael fruit extract from hydrogels across rat abdominal skin. The skin permeation profile showed the same pattern as that of the *in vitro* drug release profile. The *ex vivo* cumulative drug release from formulation F6 at the end of 24h was found to be 72.64 %.

3.3.9. Release kinetics

The release data obtained was fitted to different kinetic models to calculate release constant and regression coefficient. The obtained result is summarized in table 8 and figure 6, 7, 8 and 9. The hydrogel of batch F1 to F6 and *ex vivo* was best fitted to Higuchi model with the highest regression coefficient value indicating diffusion controlled drug release. In Korsemeyer- Peppas, the n value of batch was found to be $0.45<n=0.89$ hence we can conclude that it follows non fickian/ anomalous diffusion model.

3.3.10. Scanning electron microscopy (SEM) images of hydrogel

From the SEM image it was clear that hydrogel without the crosslinking agent maintained structure integrity and desired consistency was obtained. Hence the crosslinking agent was not used in the formulation. SEM images are shown in figure 10 and 11.

3.3.11. Wound healing activity

Figure 12 depicts the results of *in-vivo* wound healing investigations. From the figure, it could be noticed that treated group showed a time dependant increase in % wound contractions higher than that produced by the control group. These contractions were statistically significant ($p<0.001$). The formulation (F6) showed the highest % wound contraction from 11.3 ± 1.1 to 94.3 ± 1.3 from 1st day to 7th day, while complete wound closure and epithelization was observed on 14th day of wound induction. This fast and higher wound contraction rate may be ascribed to the dual effect of the drug and the polymer used. The hydrogels have a tendency to absorb wound exudates, keeping the wound region dry and speeding up the wound healing process.

4. CONCLUSION AND SUMMARY

The selected method was able to produce hydrogel with good consistency and desired properties for topical application. The prepared hydrogel had maximum swelling for 60 min. The release kinetics studies showed that it followed higuchi model, release mechanism was found to be non fickian/ anomalous behavior. The swelling property of the prepared hydrogel showed that the relaxation of polymer and diffusion of drug occurred simultaneously. The batch F6 was found to be ideal formulation hence subjected for wound healing activity, which showed complete wound healing at the end of 14 days. Therefore the study intensifies that bael fruit extract based topical drug delivery has potential to treat wounds.

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Table 1: Formula for preparation of bael fruit pulp extract loaded hydrogel Formulation.

INGREDIENTS	F1	F2	F3	F4	F5	F6
Bael fruit extract (gm)	1	1	1	1	1	1
Carbopol-940 (gm)	0.5	0.25	0.25	0.25	0.25	0.375
Guar gum (gm)	-	0.125	0.25	0.375	0.5	0.125
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s
Isopropyl myristate (ml)	1	1	1	1	1	1
Methyl paraben (%w/w)	0.1	0.1	0.1	0.1	0.1	0.1
Distilled water (upto 50 ml)	50	50	50	50	50	50

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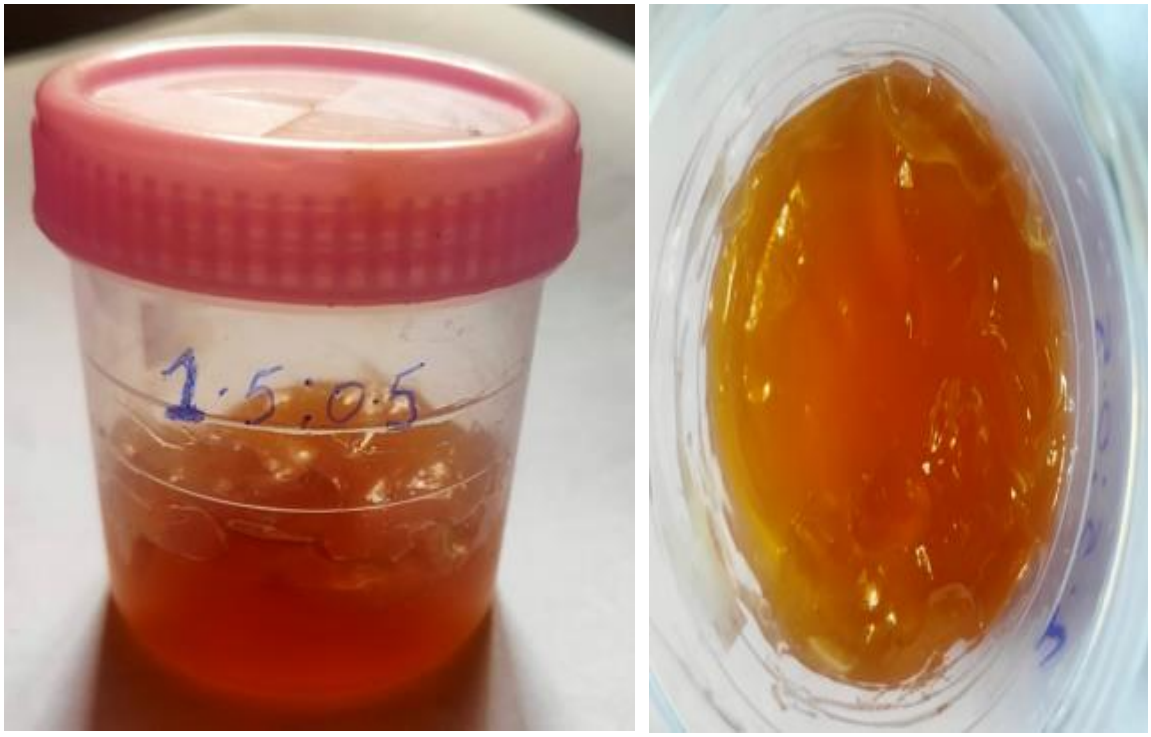

Figure. 1: Photograph of formulated hydrogel loaded with bael fruit extract.

Table 2: Preformulation studies of Bael fruit extract.

Sl. No.	Drug name	Solubility (in mg/ml)			Partition coefficient
		Water	Methanol	Hydro-alcoholic	
01	Bael fruit pulp extract	850 ± 0.12	80.18 ± 0.18	70.75 ± 0.16	3.296 ± 0.19 log P 0.571

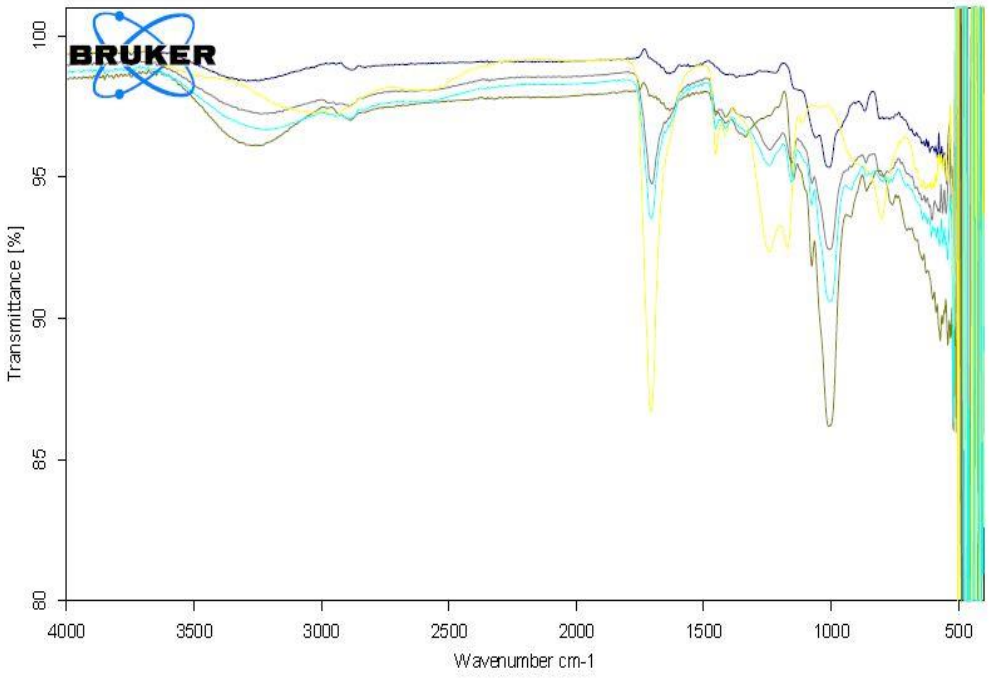

Figure. 2: FT-IR spectrum of mixture of Bael fruit extract, carbopol and guar gum.

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Table 3: Physical appearance, drug content and pH of various formulation.

Formulation code	Physical appearance	% Drug content	pH
F1	Brownish transparent	89.76 ± 0.98	3.36 ± 0.10
F2	Brownish transparent	89.82 ± 1.01	4.72 ± 0.09
F3	Brownish transparent	90.32 ± 0.88	5.17 ± 0.18
F4	Brownish transparent	90.17 ± 0.89	5.31 ± 0.24
F5	Brownish transparent	89.66 ± 0.99	5.27 ± 0.17
F6	Brownish transparent	89.80 ± 0.79	5.40 ± 0.21

Table 4: Viscosity and swelling index of various formulation.

Formulation code	Viscosity cps	Swelling Index %	
		1hr	3hr
F1	16890 ± 1123	167.39 ± 0.23	183.59 ± 0.35
F2	75730 ± 1254	132.70 ± 0.18	166.79 ± 0.82
F3	17085 ± 1618	99.06 ± 0.54	102.69 ± 0.41
F4	18043 ± 1245	61.79 ± 0.61	68.85 ± 0.65
F5	18342 ± 2010	32.57 ± 0.39	34.16 ± 0.51
F6	17525 ± 1119	150.98 ± 0.58	204.98 ± 0.32

Table 5:

Spreadability of F1 to F6 formulations.

Weights in gm	Initial cm	Spreading diameter in cm					
		F1	**F2**	**F3**	**F4**	**F5**	**F6**
10	1	5.1 ± 0.63	4.9 ± 0.36	4.1 ± 0.68	3.2 ± 0.28	2.7 ± 0.44	4.1 ± 0.19
20	1	5.2 ± 0.54	5.0 ± 0.45	4.2 ± 0.49	3.7 ± 0.96	2.8 ± 0.83	4.2 ± 0.55
30	1	5.3 ± 0.48	5.1 ± 0.81	4.4 ± 0.94	3.7 ± 0.33	2.9 ± 0.62	4.4 ± 0.68
40	1	5.4 ± 0.54	5.1 ± 0.97	4.6 ± 0.58	3.9 ± 0.48	3.2 ± 0.58	4.5 ± 0.79
50	1	5.4 ± 0.38	5.1 ± 0.25	4.6 ± 0.39	4.3 ± 0.86	3.4 ± 0.98	4.6 ± 0.29
60	1	5.4 ± 0.89	5.1 ± 0.67	4.6 ± 0.94	4.6 ± 0.37	3.6 ± 0.27	4.8 ± 0.35
70	1	5.4 ± 0.98	5.1 ± 0.87	4.6 ± 0.57	4.6 ± 0.57	3.8 ± 0.87	4.9 ± 0.49
80	1	5.4 ± 0.56	5.1 ± 0.93	4.6 ± 0.86	4.8 ± 0.19	3.9 ± 0.33	5.0 ± 0.97
90	1	5.4 ± 0.45	5.1 ± 0.29	4.6 ± 0.39	5.0 ± 0.27	4.0 ± 0.68	5.0 ± 0.59
100	1	5.4 ± 0.88	5.1 ± 0.79	4.6 ± 0.49	5.0 ± 0.49	4.0 ± 0.81	5.1 ± 0.58

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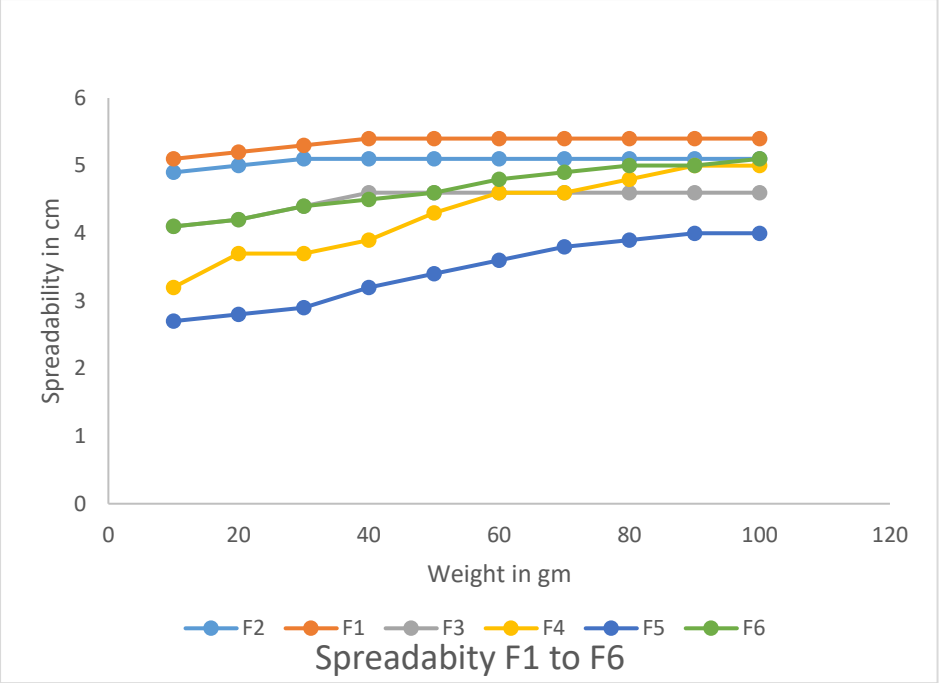

Figure. 3: Comparative spread ability of bael fruit extract loaded hydrogel of F1 to F6.

Table 6. *In vitro* drug release profile of bael fruit extract loaded hydrogel of F1 to F6.

Sl. No.	Time (h)	Cumulative percentage of drug released (%)					
		F1	F2	F3	F4	F5	F6
1	0.25	2.60	3.16	2.18	2.496	2.62	3.22
2	0.5	7.19	6.93	8.73	5.664	3.03	13.71
3	1	11.36	10.05	9.66	7.680	4.95	17.14
4	2	18.14	13.97	16.73	11.61	6.06	26.81
5	4	23.25	19.88	25.25	13.53	9.19	35.95
6	6	35.04	23.86	30.97	17.37	12.93	48.63
7	8	43.18	32.22	33.67	19.48	15.56	57.68
8	10	50.69	40.58	40.11	25.24	23.84	64.85
9	12	54.34	44.25	43.23	32.83	29.20	71.71
10	24	55.27	53.49	48.11	38.01	31.72	79.29

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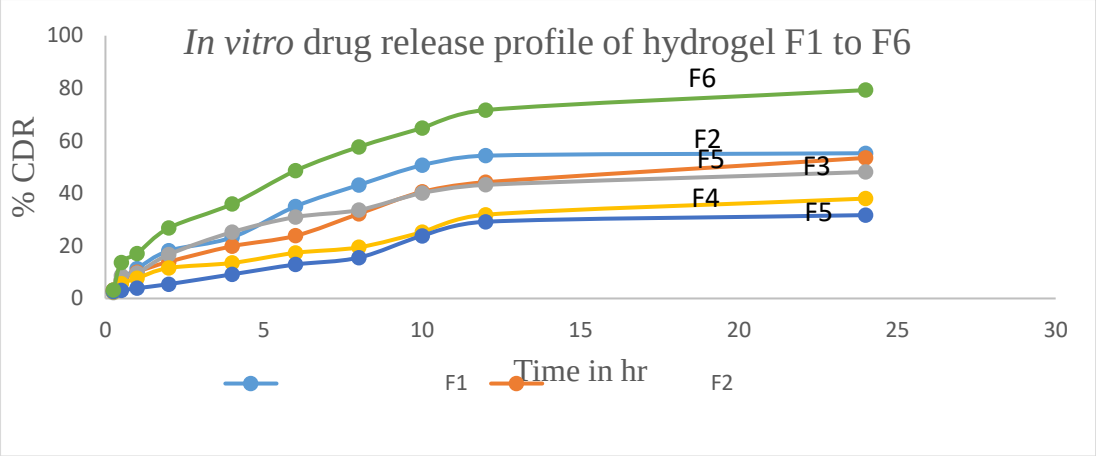

Figure. 4: Comparative *in vitro* drug release profile of bael fruit extract loaded hydrogel of F1 to F6

Table 7: *Ex vivo* permeation study profile of bael fruit extract loaded hydrogel of F6 (ideal formulation).

Sl. No.	Time (h)	Cumulative percentage of drug released (%) F6
1	0.25	3.63 ± 0.58
2	0.5	10.18 ± 0.89
3	1	16.52 ± 0.67
4	2	23.90 ± 0.99
5	4	31.69 ± 0.76
6	6	43.65 ± 0.59
7	8	52.17 ± 0.88
8	10	60.79 ± 0.72
9	12	67.55 ± 0.91
10	24	72.64 ± 0.87

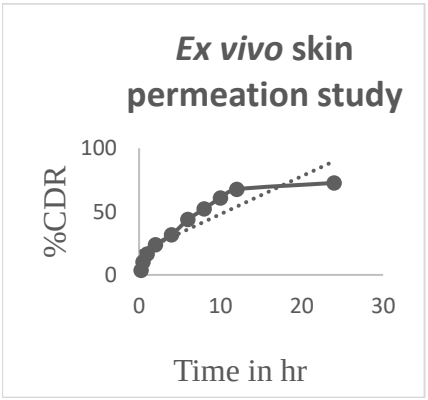

Figure. 5: *Ex vivo* skin permeation study of bael fruit extract loaded hydrogel of F6.

Table 8. Release kinetics of hydrogel loaded with bael fruit extract (F1 to F6 and *ex vivo*).

Formulation code	Release kinetics model					
	Zero order	First order	Higuchi	Korsmeyer/Peppas		Kinetics model
	R^2	R^2	R^2	R^2	n	
F1	0.9534	0.9683	0.9725	0.9559	0.7464	**Higuchi**
F2	0.9141	0.9345	0.9941	0.9662	0.5990	**Higuchi**
F3	0.9406	0.9617	0.9811	0.91225	0.7500	**Higuchi**
F4	0.8729	0.8895	0.9799	0.9435	0.5612	**Higuchi**
F5	0.9533	0.9601	0.9819	0.9766	0.5007	**Higuchi**
F6	0.9317	0.9675	0.9802	0.8920	0.7457	**Higuchi**
Ex Vivo	0.9369	0.9666	0.9439	0.9837	0.7132	**Higuchi**

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Figure. 6: Comparative Zero order release kinetics of bael fruit extract loaded hydrogel F1 to F6 and *ex vivo*.

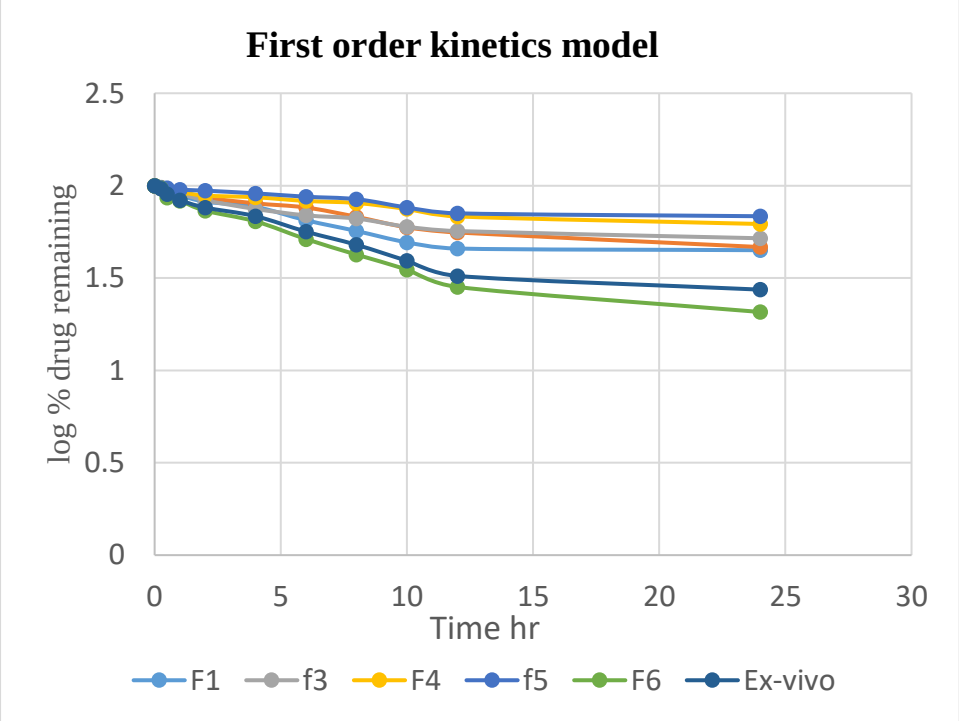

Figure. 7: Comparative First order release kinetics of bael fruit extract loaded hydrogel (F1 to F6 and *ex vivo*).

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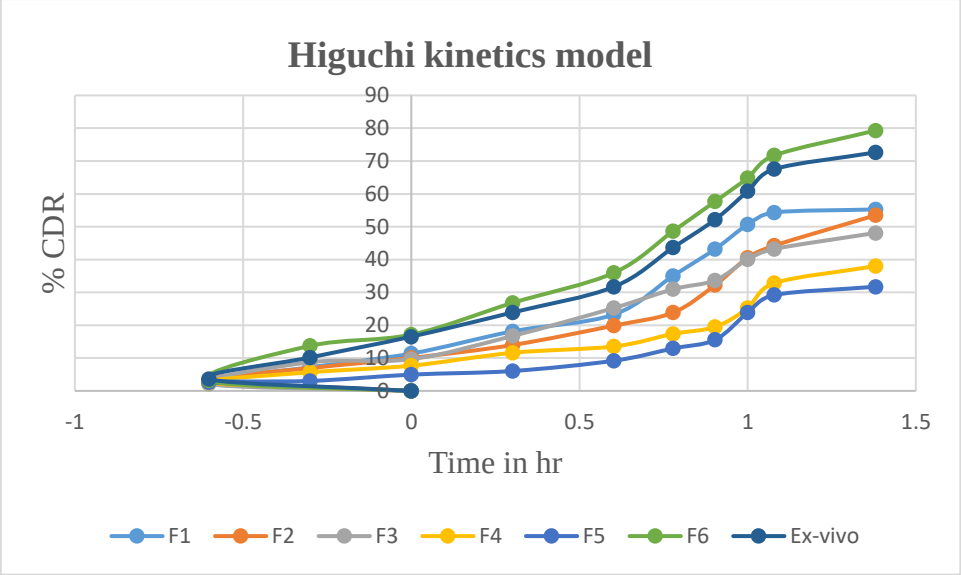

Figure. 8: Comparative Higuchi release kinetics of bael fruit extract loaded hydrogel (F1 to F6 and *ex vivo*

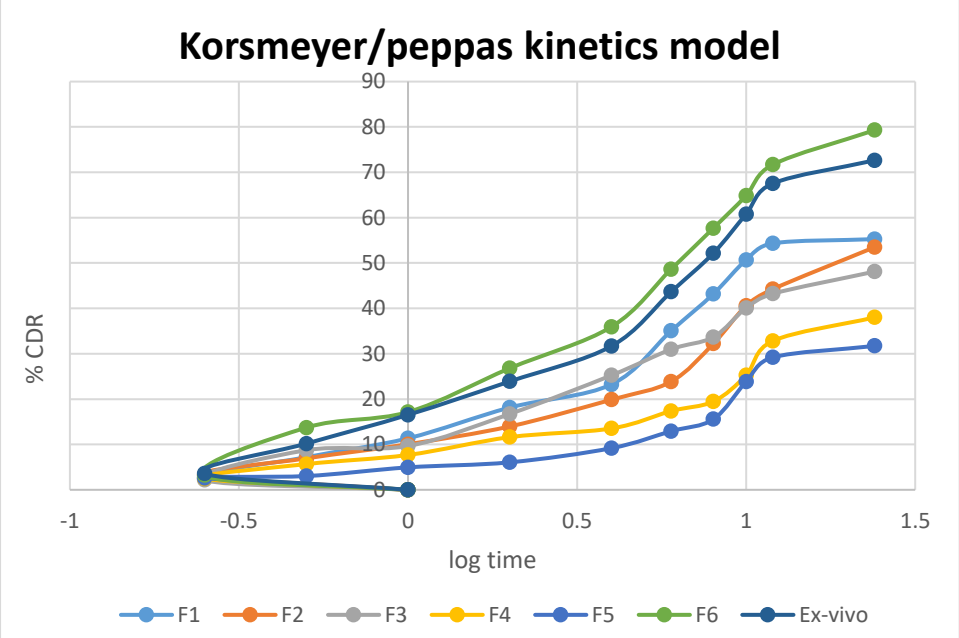

Figure. 9: Comparative korsmeyer/peppas release kinetics of bael fruit extract loaded hydrogel (F1 to F6 and *ex vivo*).

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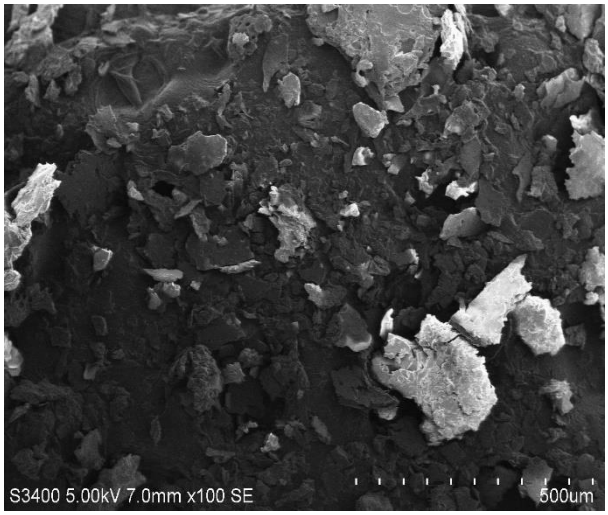

Figure. 10: SEM image of hydrogel cross-linked with NaOH

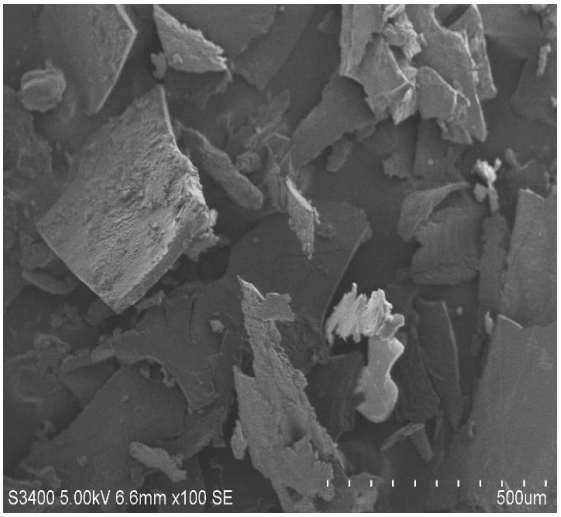

Figure. 11: SEM image of hydrogel without cross linking.

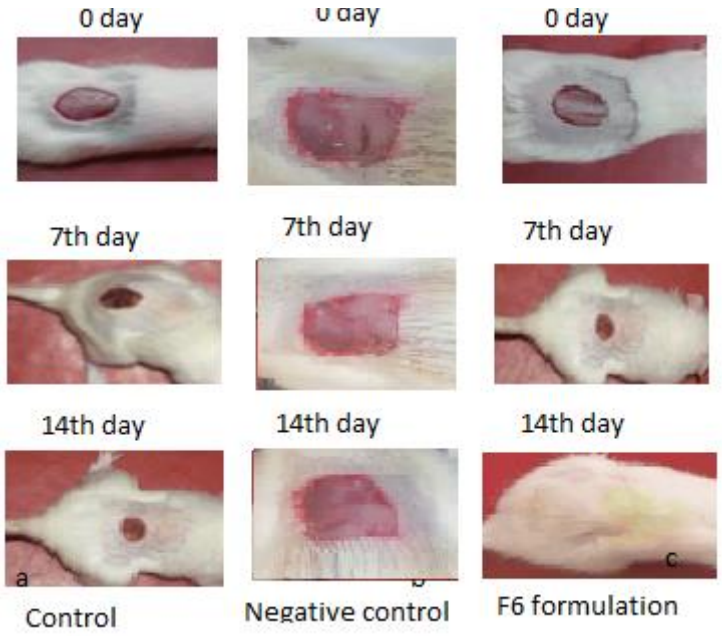

Figure 12: Wound healing activity of Bael fruit extract a) control group b) negative control and c) F6 formulation

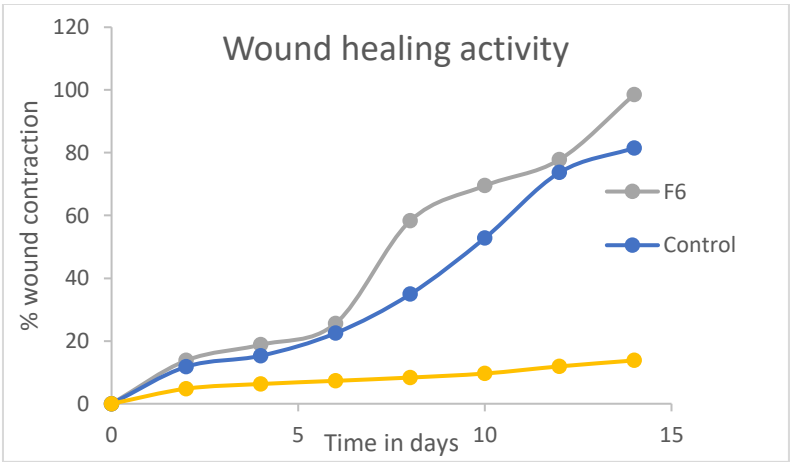

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Figure 8: Effect of control, negative control and F6 formulation on percentage of wound contraction of infected wound.