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Formulation and Evaluation of Biodegradable Film Containing Extract of Centella Asiatica for Wound Healing

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Abstract : The main objective of the present investigation was formulation and evaluation of biodegradablefilm containing extract of centella asiatica for wound healing activityThin films have been generally used around the globe for tissue repair and healing of wounds. The solvent casting method used for preparation of films was simple, reproducible and rapid. The formulated films good tensile strength, moisture absorption, and folding endurance. Evaluation parameter of this study it is the appearance, thickness, percent moisture content, folding endurance, *In- vitro In- Vivo* studies. The result shows appearance transparent and odour pleasant, thickness of optimized batch is standard size, folding endurance & tensile strength it is 300±11&21.92.*In- Vivo* studies shows wound healing of that the optimized formulation comparing with control & standard it gives better result control 7.2±0.90, standard 0.84±0.42, test 0.87±0.37 .Conclusion of this study the biodegradable film was to deliver a drug in a sustained manner for an extent period of time to reduce frequency of application and to improve bioavailability for treating wound infections.
Keywords : Biodegradablefilm, *Centella asiatica*, solvent casting, Gelatin: sodium alginate: ethylene glycol, healing of wounds.

Introduction

The goal of any drug delivery system is to provide a therapeutic amount of drug to proper site in the body to achieve and maintain therapeutic concentration within range and to show pharmacological action with minimum incidence of adverse effects. To achieve this goal one should maintain dosing frequency and suitable route of administration.*Centella asiatica*(L.) belongs to the family Apiaceae (Umbelliferae) (Fig. 1). This herb is indigenous to the Indian subcontinent, Southeast Asia, and wetland regions of the Southeastern USA. It is a popular medicinal plant in several traditional medicines, especially in wound healing[1]. Based on numerous studies, the biologically active ingredients are believed to be triterpenes, namely, asiatic acid, madecassic acid, asiaticoside and madecassoside[2, 3]. Due to its ability to stimulate collagen synthesis, centella has been used in skin care products for restoring skin firmness, elasticity and improving skin appearance. Traditional dressings which had their main function as absorbing wound exudate, and led to formation of crust on the wound surface

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with remarkable scarring have been widely replaced by modern dressings aiming to improve healing by handling wound fluid in a way that prevents accumulation of excess exudate while maintaining a certain degree of moisture, and thereby enhancing the chance of obtaining new skin tissue without scarring[4].it not only increase the rate of healing but also maintains the aesthetic conditions, play a significant role in wound care management[5, 6].On the basis of condition wound could be dividing in to two types: one is chronic wounds and other is burn wound and sepsis [7, 8]. Wound healing is specific biological process related to the general phenomenon of growth and tissue regeneration. The entire process of wound healing is ordered cascade of events, which can be divided into four distant but over lapping phase of homeostasis, inflammation, proliferation and maturation[9, 10, 11]

Figure No.1: ***Plant of Centella asiatica***

Materials And Method

Materials

Centella asiatica was obtained from Mankarnika Aushadalya, Pune. Gelatin, Sodium alginate, Liquid paraffinwere a Gift sample from S.D. Lab chemical centre Mumbai. Ethylene glycol MERK limited Mumbai. All other chemicals and ingredients were used for study are of Analytical grade.

Methods[12]

Preparation of Biodegradable film by film casting Method

The film was prepared according to the casting technique. For the preparation of biodegradable film, the gel of gelatin and sodium alginate were prepared in water in separate beaker. Then both gels were mixed, then add drug extract in the gel then add plasticizer in that mixture and poured in to petrieplates which is already greased with liquid paraffin and kept for drying in shade.

Formulation design of Biodegradable films containing centella asiatica extract Optimization of formula

For optimizing Formula for Biodegradable films the quantity of excipient were changed and the evaluation test for Biodegradable films are carried out.

Full Factorial Design:

A factorial design is used to evaluate two or more factors simultaneously. The full Factorial design is a technique that allows identification of factor involved in a process and assesses their relative importance. The advantages of Factorial design over on factor at a time experiments are that they are more efficient and allow interaction to be detected.. A factor is simply a categorical variable with two or more values referred to as levels. Construction of factorial a design involves the selection of parameters and the choice of response. A study, in which there are 2 factors with 3 levels, is called 3^2 Factorial Design.A 3^2Full Factorial Design (FFD)

was constructed where the amount of Gelatin (X_1), sodium alginate (X_2) and were selected as a factors. High and low levels of each factor.

Table No. 1Full Factorial Design$3^{2=}$ 9 Experiments

Formulations	X_1	X_2
F_1	+ 1	+ 1
F_2	0	0
F_3	-1	- 1
F_4	+1	0
F_5	0	+1
F_6	- 1	+ 1
F_7	+ 1	-1
F_8	0	- 1
F9	-1	0

Table No. 2 Amount of variables in 3^2 Full Factorial Design

Coded Values	Actual Values	
	Gelatin(ml)	Sodium alginate(ml)
-1	9.5	0.7
0	10	0.8
+1	10.5	0.9

Table No. 3Composition of factorial designed batches

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug (gm)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Gelatin (ml)	10.5	10	9.5	10.5	10	9.5	10.5	10	9.5
Sodium alginate(ml)	0.9	0.8	0.7	0.8	0.9	0.9	0.7	0.7	0.8
ethylene glycol (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Preparation of Biodegradable film containing extract of Centellaasiatica:

The film was prepared according to the casting technique. For the preparation of biodegradable film, the gel of gelatin and sodium alginate were prepared in water in separate beaker. Then both gels were mixed, then add drug extract in the gel then add plasticizer in that mixture and poured in to petriplates which is already greased with liquid paraffin and kept for drying in shade.

Evaluation Parameters[13,14]

Pre-formulation Studies

Drug and Excipient interaction:

Visual observations:

The samples subjected to drug-excipient compatibility studies were assessed for any visual changes. The samples were observed for change in colour and nature.

IR Spectroscopy (FTIR – 410, Jasco, Japan).

IR spectroscopy is one of the most powerful analytical techniques, which offers the possibility of detecting chemical interaction. The IR spectra analysis of Centellaasiatica, Sodium alginate, Gelatin, was successfully done to be checking the compatibility of the drug with excipient. IR spectra of Centellaasiatica and formulation were recorded by using Fourier transform Infrared spectrophotometer. A baseline correction was done by blank background measurement.

DSC

Studies were performed on the pure drug and formulation in order to study the interaction between drug and polymer in the formulations to investigate the thermal behavior of formulation mixture.

Evaluation ofBiodegradable film:

Appearance:

Appearance was determined by using visual examination.

Thickness:

Thickness of film was measured at five different randomly selected spots using micrometer screw gauge. Weight of film is helpful to ensure that the film contains the proper amount of excipient and API; hence film weight should be nearly constant. Thickness of film is directly related to the accuracy of dose in the film.

Percent moisture (content) loss:

The prepared film was weighed. Then kept in to dessicator containing fused calcium chloride at room temperature for 24 hrs. After 24 hrs the films was reweighed and determine percent moisture content was calculated by using following formula.

$$\text{Percent moisture (content) loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

Percent moisture absorption (uptake):

The prepared film was weighed. Then kept in to dessicator containing saturated solution of potassium chloride at room temperature for 24 hrs. After 24 hrs the films was reweighed and determine percent moisture content was calculated by using following formula.

$$\text{Percent moisture (content) loss} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 10$$

Tensile strength:

Tensile strength is maximum stress applied to a point at which the film breaks. In this test the film was tied between two clamps and the one end of clamp is directly attached to pan through pulley. The stress was applied to the film by putting load in the pan and finally the reading of load at failure is noted. Tensile strength was calculated by formula,

$$\text{Tensile strength of film} = \frac{\text{load at failure}}{\text{film thickness} \times \text{film width}} \times 100$$

Folding endurance:

Folding endurance of the film is essential to study elasticity of the film during storage. And which is needed to handle the film easy, comfortable and for secured application of film on the wound. It was determined by repeatedly folding the film at same place till it breaks or folding up to 300 times. This is considered to reveal good film properties.

Surface ph:

It is determined by keeping film in to 15 ml of water for 1 hr. Then Ph was measured by suing Ph meter.

In- Vivo studies for wound healing activity by using suitable animal model:

Wound healing activity:

Animals required

Species/ Common name – Wister rats male and female 200 to 300gm (n= 18)

a.	Age	More than 2 month.
b.	Gender	Either Male and Female

The rats were divided into three groups namely,

1. **Group I:** Control
2. **Group II:** Standard
3. **Group III:** Test (Biodegradable films)

Procedure:

The rats were divided into three groups namely control, Standard, and test (formulation) on the day of experiment the whole rats were anesthetized. A full thickness of the excision wound with circular area of 176 mm^2 (width 1.5 cm and depth 0.2 cm) was made on the shaved back (dorsal thoracic region) of the rats. The wounding day was considered as day 0 the wound were treated with topical application. The wound contraction were measured by a tracing paper on the wounded margin and calculated as percentage reduction in wound area. The wound were monitored and the area of wound size was measured on 3,6,9,12,15 and 18 th of post wounding day.

The percentage of wound closure was calculated using the following formula:

% wound closure

$$= \frac{\textbf{Wound Area on Day'0' – Wound Area on Day 'n'}}{\textbf{Wound Area on Day '0'}} \times 100$$

Where n = number of days

Results and Discussion-

Drug and Excipient interaction:

Visual observations: nochange in colour and nature

Colour: Light Green

Odour: Pleasant

IR Spectroscopy

The authentication of Centellaasiatica was done fromMankarnikaAushadalya, Pune.And it was confirmed that the procured after were of Centellaasiatica.

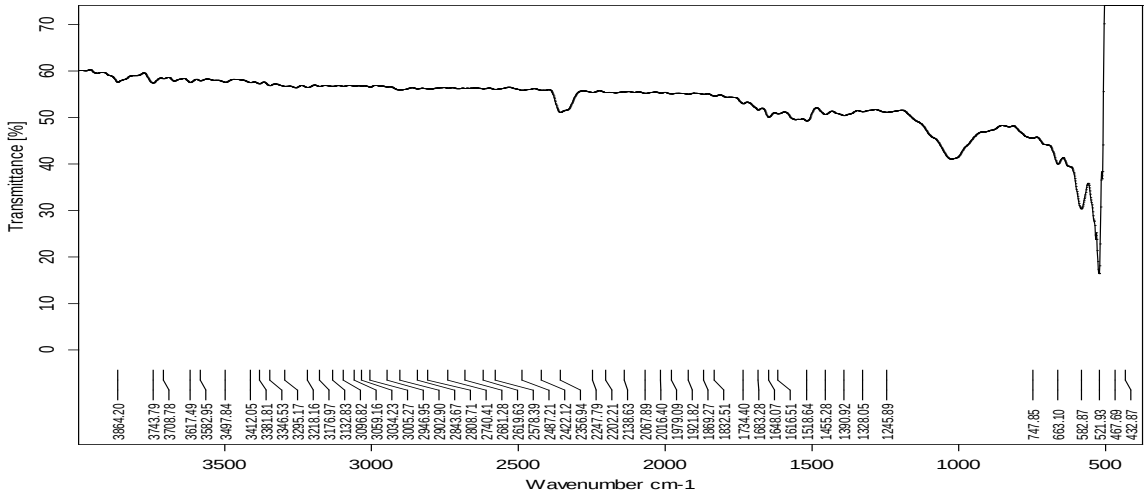


Figure No.2. FTIR spectrum of Centella asiatica

DSC studies were performed on the pure drug and formulation in order to study the interaction between drug and polymer in the formulations to investigate the thermal behavior of formulation mixture.

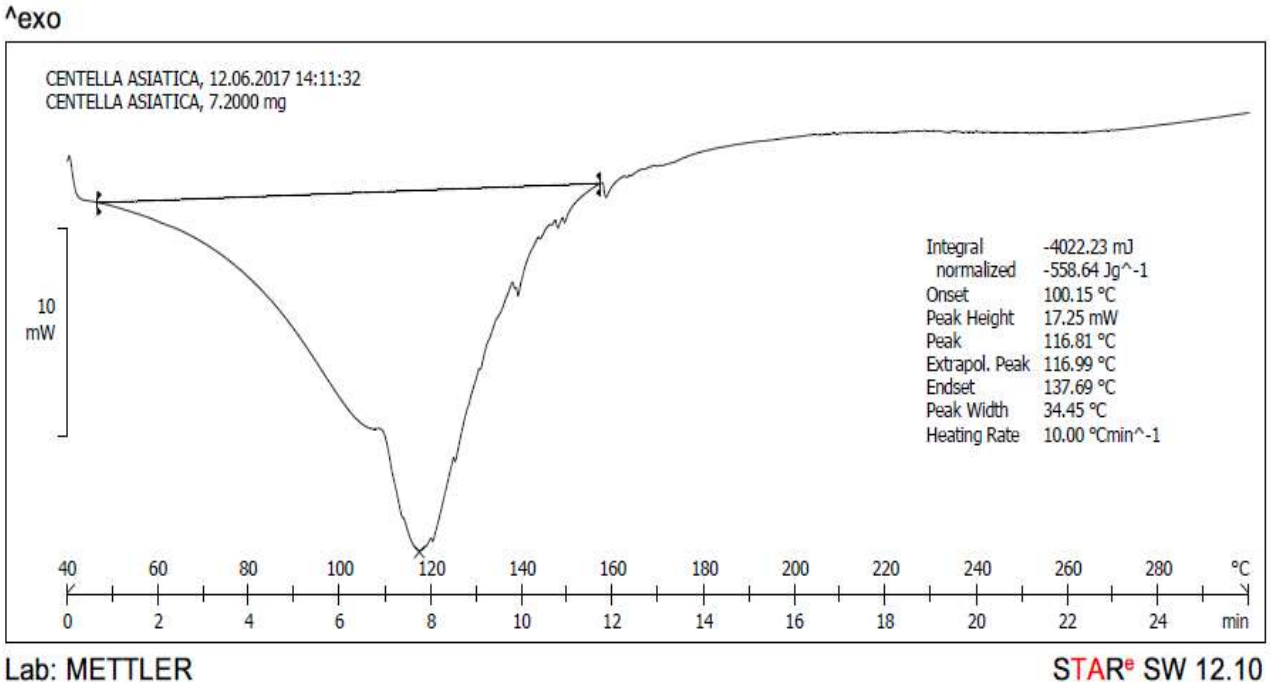


Figure No.3.DSC Thermogram of centella asiatica extract

In DSC thermogram of centella asiatica extract endothermic peak was observed at 116.81ºC

Sr. No.	Wave number (cm^{-1})	Ranges	Functional Groups Associated
1	2356.94	2100-2400	C=N
2	1616.51	1500-1700	N-H
3	521.93	500-600	C-Br
4	1734	1600-1900	C=O

Table No. 4 Preformulation study:

Batch no.	Appearance	Weight(gm)	Thickness (µm)	Folding endurance	Tensile strength (%)
F1	Transparent	1.40± 0.005	0.57± 0.023	327±52	17.54
F2	Transparent	1.36± 0.011	0.57± 0.023	298±17	18.42
F3	Transparent	1.30±0.011	0.57±0.023	306±15	19.29
F4	Transparent	1.39± 0.005	0.57±0.023	312±10	21.09
F5	Transparent	1.36± 0.005	0.57±0.023	306±40	16.66
F6	Transparent	1.32± 0.005	0.57±0.023	305±21	20.17
F7	Transparent	1.37± 0.011	0.57±0.023	300±11	21.92
F8	Transparent	1.36± 0.011	0.57±0.023	326±20	18.42
F9	Transparent	1.32± 0.005	0.57±0.023	323±15	21.05

Table No. 5 Evaluation of formulation

Batch no.	Surface ph	Moisture loss (%)	Moisture uptake (%)
F1	6.1±0.05	5.21± 1.31	35.27±0.17
F2	6.3±0.05	4.07±0.45	44.35±1.12
F3	6.1±0.05	3.44± 0.47	44.35± 1.12
F4	6.7±0.05	5.03± 0.46	35.25±1.44
F5	6.1±0.05	3.52±0.45	38.40± 0.72
F6	7.03±0.05	4.76±0.83	36.36± 0.76
F7	6.0±0.05	2.72± 0.43	32.23± 1.08
F8	6.8±0.05	4.61± 0.80	36.26±0.42
F9	6.1±0.05	3.12± 0.02	43.93±1.01

Discussion:

Visual Appearance:

The prepared films of Gelatin and Sodium Alginate were observed visually. They were found to be transparent. The films had smooth surface without any scratches.

Weight of Films:

The films of all formulation were tested for weight variation. The average weight of individual formulation is given in table 13. The weight of all prepared films, ranged between1.30gm-1.40gm the variation in average weight of all formulations might be due to change in film former and polymer concentration.

Thickness of Films:

Thickness of films was determined by using screw gauge micrometer. The average thickness value of all prepared batches is given in table 13. The thickness of formulation was found to be increased in batch F1, F4, F7 it is mainly because of increasing concentration of Gelatin. All the formulations contained different amount of polymer concentrations. When the concentration of Gelatin was increased, thickness was found to be increased.

Folding Endurance of Films:

The formulation F7 showed highest folding endurance as compared to other formulations. As the concentration of Gelatin polymer was increased, the folding endurance of film was found to be increased to a certain extent Increasing concentration of polymer increased the flexibility at certain extent. To prevent that plasticizers are employed in very low concentration. The folding endurance of all prepared films ranged between 298-353.

Tensile Strength of Films:

The average tensile strength of individual formulation is given in the table 13. As the concentration of Gelatin polymer was increased, the tensile strength of film was found to be increased. F5 formulation containing highest concentration of sodium alginate polymer showed lowest tensile strength as compared to other formulations. The decrease in tensile strength was might be due to weakening of bond linkage between the polymer chains

Percent moisture Absorption:

The % moisture absorption study gives idea about the stability of film. More the moisture absorption property of the film, the film will easily get degraded. The results of % moisture absorption are given in table 13. From table it was found that, as the concentration of sodium alginate in film decreases moisture absorption of film increases.

Percent moisture content (loss):

The results of % moisture content are given in table 13. From table it was found that, Higher the concentration of sodium alginate and gelatin in film shows increases moisture content of film. This might be due to hydrophilic nature of polymer. The % moisture content of all prepared films Ranged between 2.72- 5.21.

Surface Ph:

The results of surface Ph content are given in table 13. From table it was found that, Higher the concentration of sodium alginate and minimum concentration of gelatin in film shows increased surface ph of film. The surface Ph of all prepared films Ranged between 6.1-7.

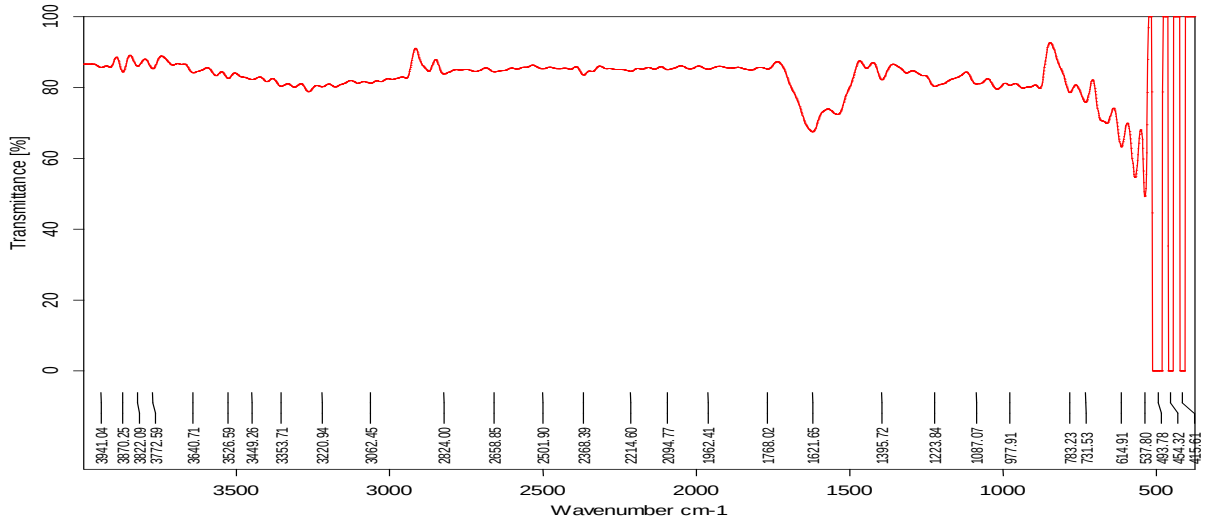


Figure No. 4.FTIR spectrum of Physical mixture

Table No. 6FTIR spectrum of Physical mixture

Sr. No.	Wave number (cm^{-1})	Ranges	Functional Groups Associated
1	1621.65	1640-1550	N-H (Bend)
2	1395.72	1680-1600	CH3 Alkanes (Bend)

3	614.19	785-540	chloride
4	726.96	650-1000	Alkenes (out of plane bend)
5	521.93	500-600	C-Br

Figure No. 5.FTIR spectrum of Centella asiatica extract containing film

Table No.7FTIR spectrum of Centella asiatica extract containing film

Sr. No.	Wave number (cm^{-1})	Ranges	Functional Groups Associated
1	3475.37	3100-3500	N-H (Stretch)
2	1605.51	1680-1600	C=C
3	1449.28	1335-1550	N=O
4	726.96	650-1000	Alkenes (out of plane bend)

DSC thermogram of Centella asiatica extract containing film

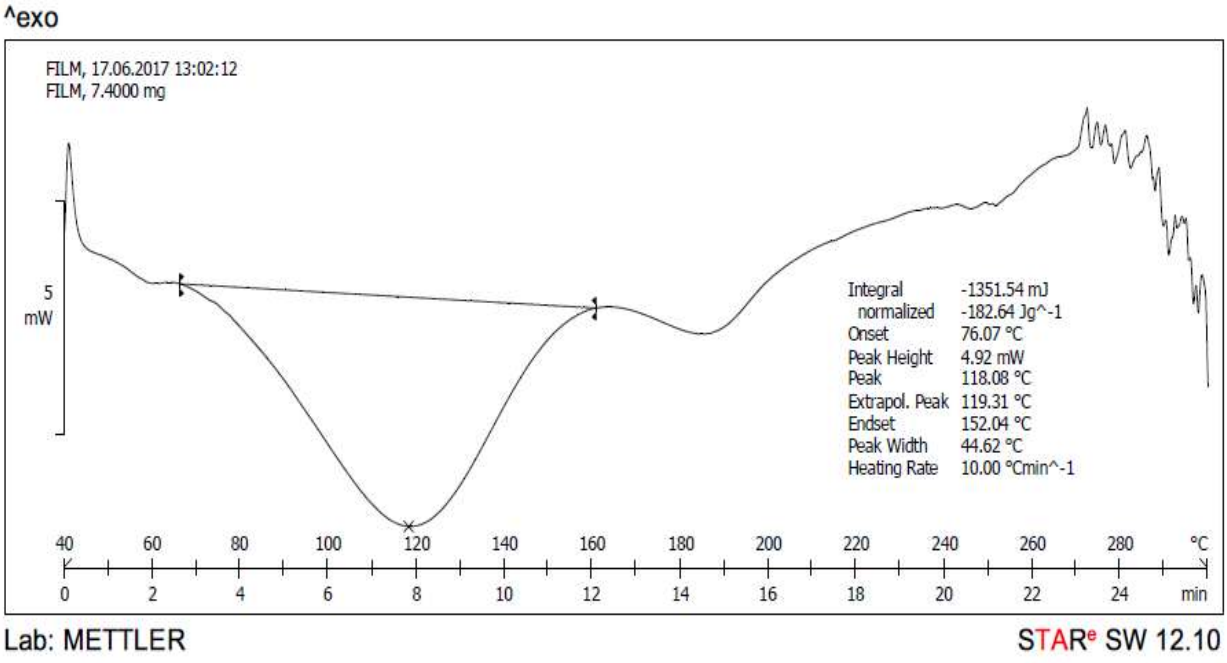


Figure No.6.: DSC thermogram of Centella asiatica extract containing film

In DSC thermogram of Centella asiatica extract containing film endothermic peak was observed at 118.8ºC. The DSC thermogram of drug and formultion compared with each other it reflected that both shows endothermic. The study indicated that drug has not under go any chemical interaction with the polymer.

Stability studies:

The stability studies of formulated Films were carried out 40/75(ºC/RH) and at room temperature for one month. The effect of temperature, humidity and time on the physical characteristics of the Film was for assessing the stability of the prepared formulations. The stability studies were carried out when the room temperature was 20 to 25º C. The Results were shown in Table no. 8.

Table No. 9 FTIR spectrum of Centella asiatica extract containing film

Duration	Visual appearance	weight	Thickness	Folding endurance
0 Days	Transparent	1.32± 0.005	0.57±0.023	323±15
30 Days	Transparent	1.31±0.005	0.57±0.023	301±12.58

Table No. 10 FTIR spectrum of Centella asiatica extract containing film

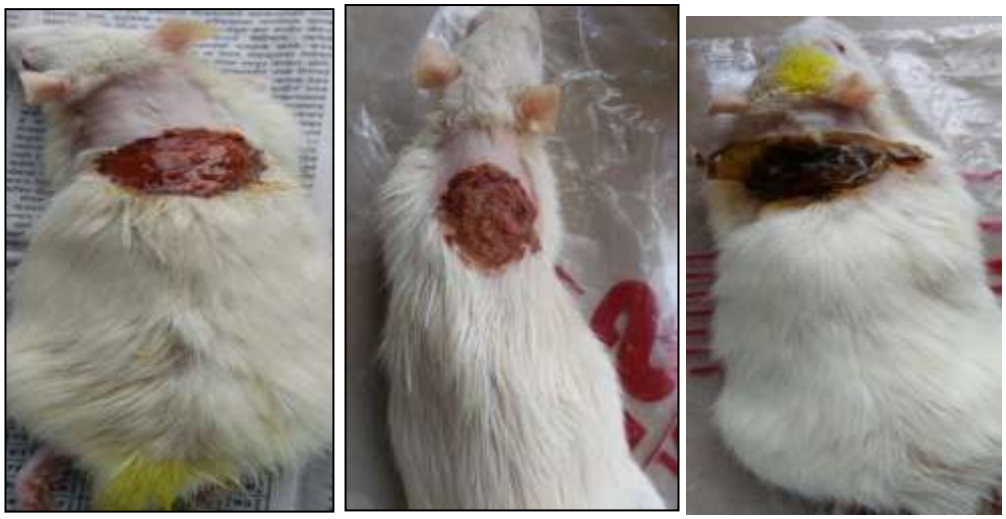
Duration	Tensile strength	Moisture uptake (%)	Moisture content(%)	Surface Ph
0 Days	21.05	43.93±1.01	3.12± 0.02	6.1±0.05
30 Days	20.38	41.53±1.19	2.83±0.25	6.2±0.1

***In-vivo* wound healing activity in animal model**:

Day 0

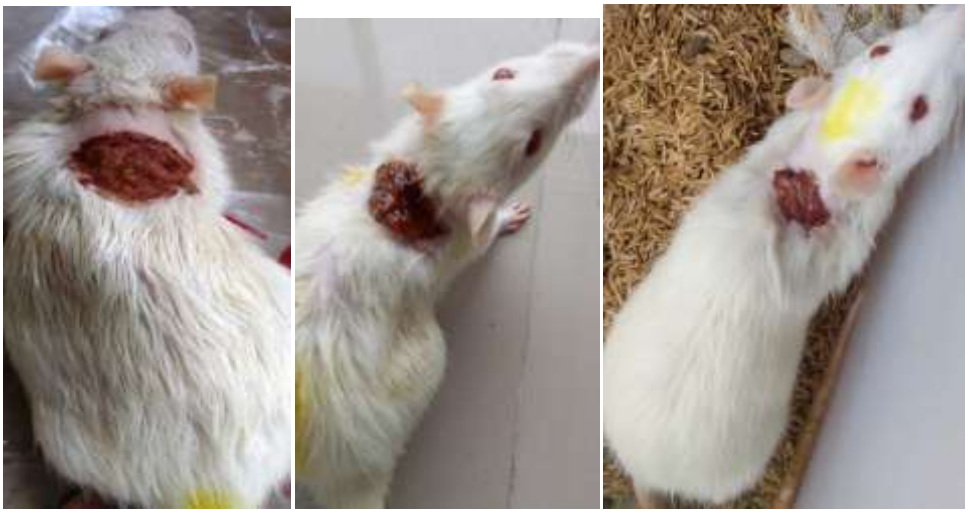
Control **Standard** **Test (Formulation)**

Day 3



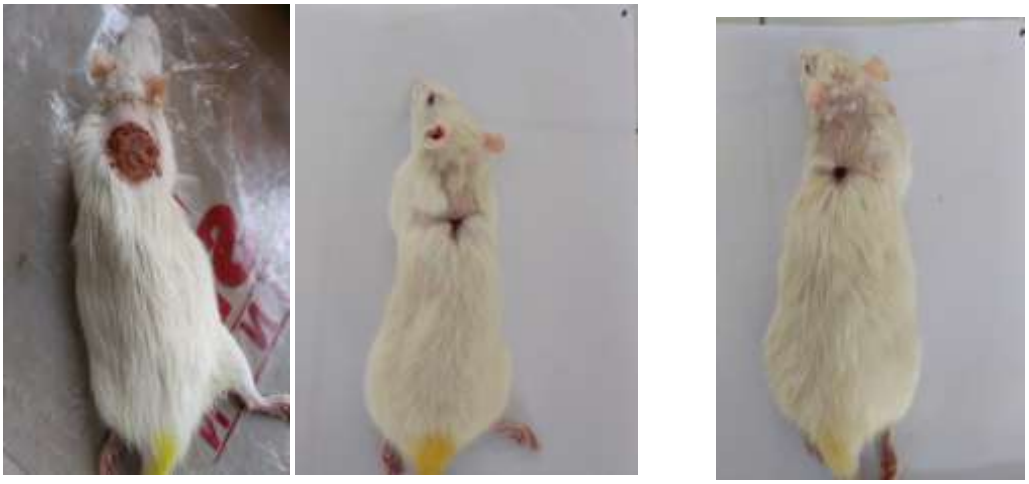
Control **Standard** **Test (Formulation)**

Day 6



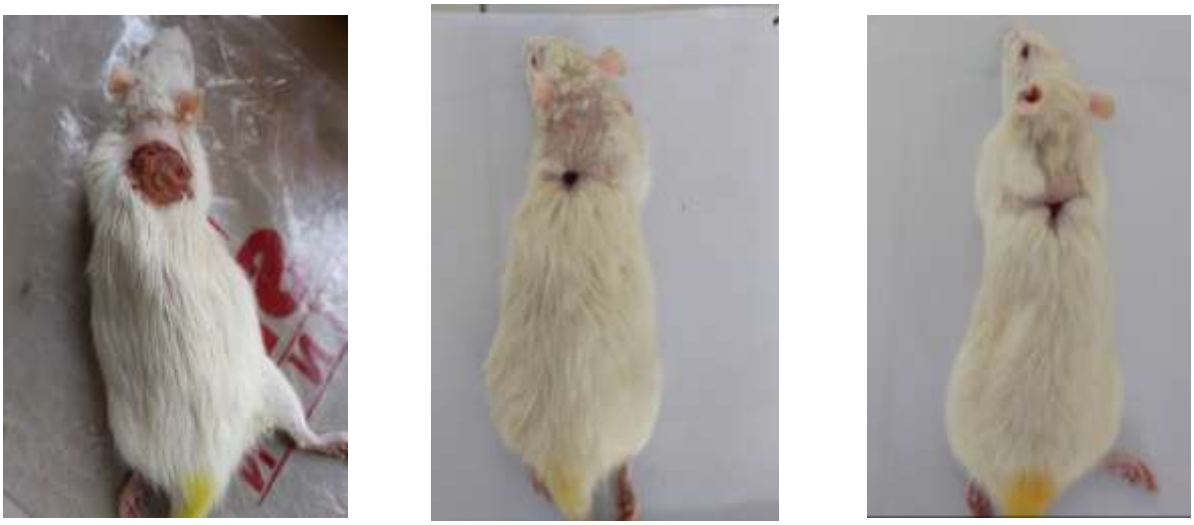
Control **Standard** **Test (Formulation)**

Day 9



Control **Standard** **Test (Formulation)**

Day 12



Control **Standard** **Test (Formulation)**

Figure No. 6.Effect of herbal Film on wound size at different day's interval

Table no. 11 Effect of herbal Film on wound size at different day's interval

Groups	**Wound size area in mm^2(mean± SEM)**				
	0 day	**3 day**	**6 day**	**9 day**	**12 day**
Control	9.22±1.42	8.55±1.29	7.9±1.04	7.6±1.02	7.2±0.90
Standard	9.22±0.77	5.34±1.37	2.78±0.62	1.9±0.82	0.84±0.42
Test	9.23±1.09	5.70±0.97	3.89±0.58	1.75±0.36	0.87±0.37

Conclusion

In the present work Centellaasiatica extract was dispersed in films formed by gelatin and sodium alginate as the polymer. The thought behind developing a biodegradable film was to deliver a drug in a sustained manner for an extent period of time to reduce frequency of application and to improve bioavailability for treating wound infections. The solvent casting method used for preparation of films was simple, reproducible and rapid. The formulated films good tensile strength, moisture absorption, and folding endurance. The extra advantage of film was that these are easy to apply and compatible with skin, non-irritant, non-toxic. This study concluded that the biodegradable film with Gelatin: sodium alginate: ethylene glycol (9.5:0.8:0.5) ratio was more efficient. As compared to conventional formulation these biodegradable films are expected to adhere to the skin for longer period of time; gradually releasing drug over the time and also causes wound healing without formation of scars. Stability study showed no significant change in various parameters of optimized formulation and hence indicated stable formulation.

Conflict of Interest - No

Acknowledgement

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