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FORMULATION AND EVALUATION OF TOPICAL GEL OF SOME PLANT ENZYMES USING CHITOSAN BIOPOLYMER

MAHENDRA C. GUNDE[*1,2], RITU M. GILHOTRA[2] AND NIKHIL D AMNERKAR[3]

[1]Kamla Nehru College of Pharmacy, Butibori, Nagpur, Maharashtra, India

[2]Suresh GyanVihar University, Mahal, Jagatpura, Jaipur, Rajasthan, India

[3]Adv.V.R.Manohar Institute of Diploma in Pharmacy, Wanadongari, Nagpur, Mahrashtra, India

***Corresponding author:** E-mail: - mgunde@gmail.com Tel: + 919545500995 .

ABSTRACT

The concept of developing formulations from plant products in indigenous medical system is much older. Interest in biomedical applications of natural biopolymer is growing fast due to their biocompatibility and biodegradable properties. In present study, we use chitosan the universally accepted nontoxic N-de-acetylated derivative of chitin as a gelling agent. Papain, a proteolytic enzyme extracted from *Carica papaya* and bromelain, mixture of proteolytic enzymes derived from *Ananus cosmosus* were used as an active ingredient. Furthermore, they all show many pharmacological activities including skin disorders. Formulations were evaluated for pH, viscosity.

Keywords: Chitosan, *Carica papaya*, *Ananus cosmosus*

INTRODUCTION

Topical gel is an effective and targeted therapy for local dermatological disorders as it provides suitable delivery of drugs because they are less greasy and easily washable from the skin [1]. It also avoids first pass metabolism, gastrointestinal irritation and metabolic degradation as in oral route of drug administration [2]. Chitosan is a polysaccharide obtained by partial deacetylation of chitin from crustacean shells such as crab and shrimp [3]. As compare to all other biodegradable polymers having a monograph in a pharmacopoeia, chitosan is the only one having a cationic characters

which make it unique. Anionic nature of mucus membrane interacts with cationic group of chitosan and achieves mucoadhesion which is essential for its topical use [4-6]. Papain, a proteolytic enzymes obtained from latex secreted by specialized lactifiers cells of papaya plant. It shows anti-inflammatory, antibacterial and antioxidant activity and also shows synergistic effect with chitosan in wound healing [7]. Bromelain, a complex mixture of proteinases obtained from pineapple plant. It shows beneficial therapeutic effects including anti-inflammatory, antioxidant etc. It has been used alone or in multi-enzyme preparation [8, 9]. The present study was aim to formulate and evaluate the topical gel formulation containing papain and bromelain with chitosan as gelling agent.

MATERIALS AND METHOD

Material

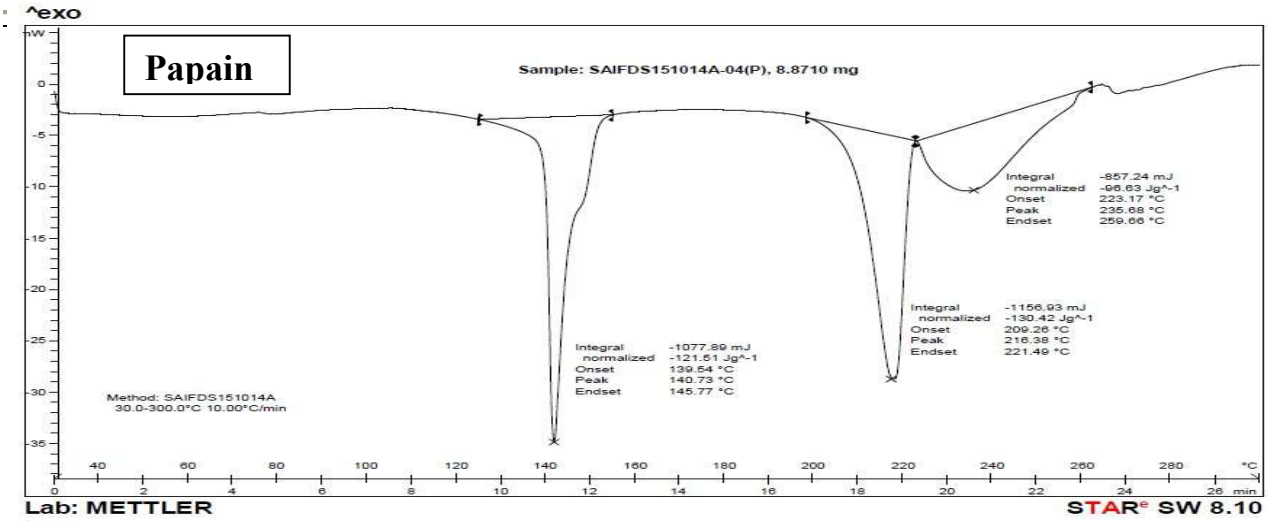
Chitosan purchased from Sigma Aldrich, Mumbai, India. Papain kindly provided as gift sample by Advanced Enzymes Technology Ltd. Thane (W), India and Bromelain purchased from Prisha Herbals, Indore, India. All other ingredients used in present study were of analytical grade.

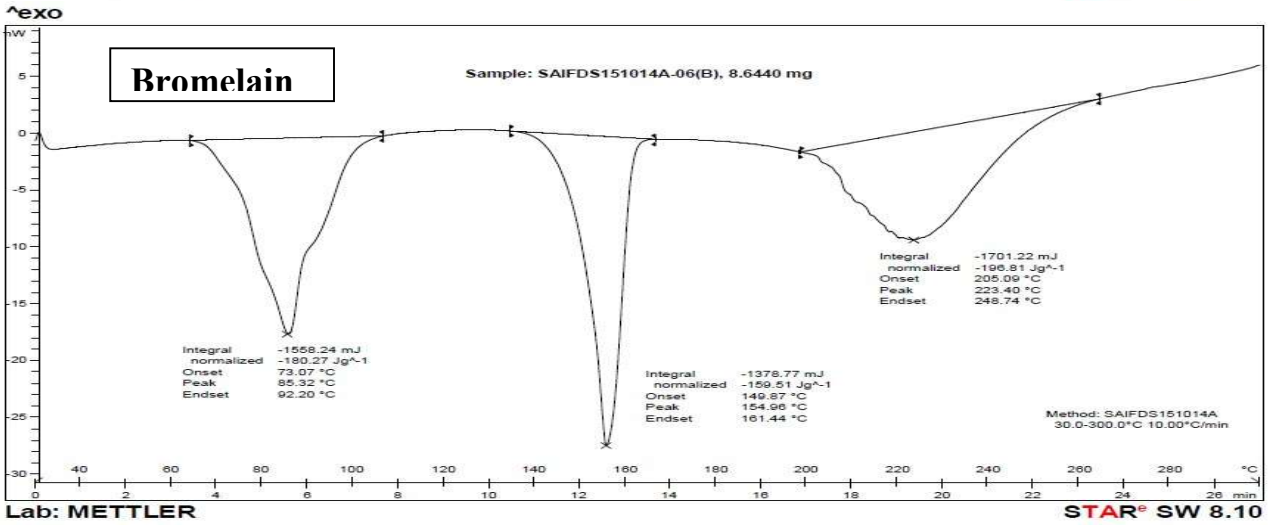
Investigation of physicochemical compatibility of plant enzymes and polymer

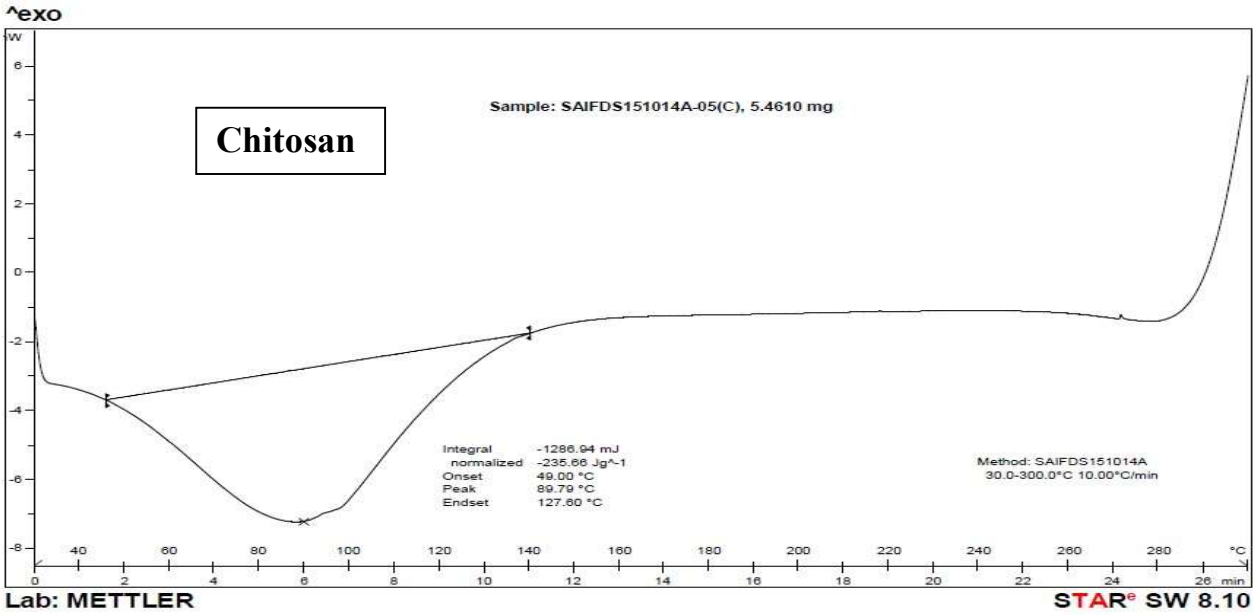
The physicochemical compatibility between papain, bromelain and chitosan used in gel formulations was studied by using Differential Scanning Calorimetry (DSC). The spectra were recorded using DSC in temperature range 30-300 ^{0}C and compare.

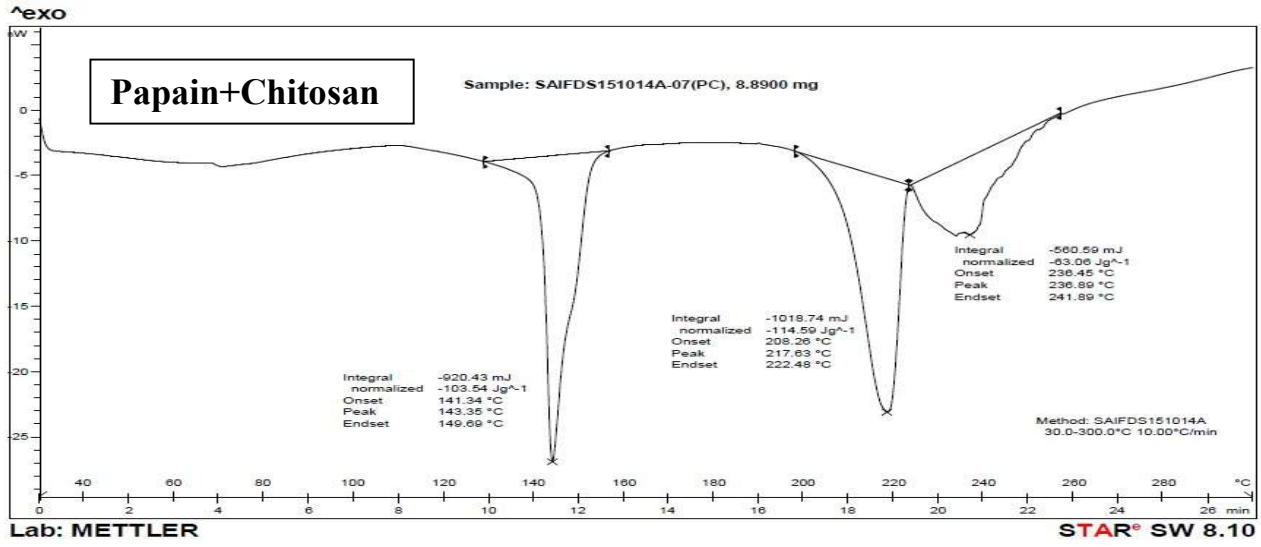
Preparation of formulation

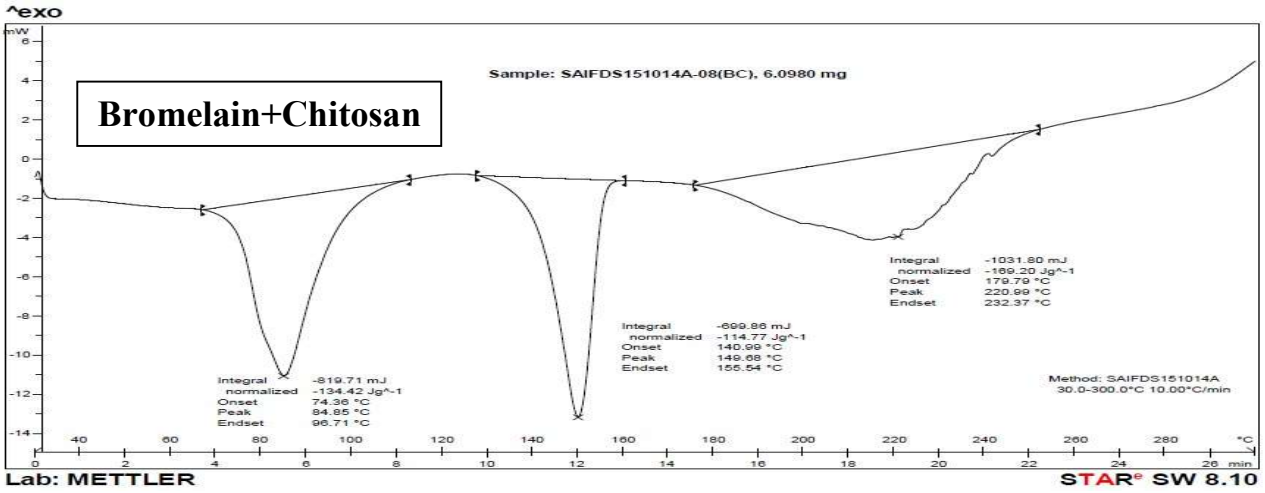
Empty chitosan gels were prepared by adding varying concentration chitosan into half of water containing glacial acetic acid (1%v/v) stirred it slowly. After the swelling remaining amount of water was added and mixed properly, methyparaben (0.1%w/w) was added as preservative. The pH was adjusted up to 6.8-7 by adding triethanolamine with continuous stirring until homogeneous gel was formed. Final volume was made up to 100 ml by adding distilled water. All the samples were allowed to equilibrate for at least 24 hours at room temperature and sonicate to remove air bubbles prior to performing rheological measurement. The same method was followed for preparation of gel containing plant enzymes [10, 11]. (Table-1).


^exo
Papain
Sample: SAIFDS151014A-04(P), 8.8710 mg
Integral -857.24 mJ
normalized -96.63 Jg^-1
Onset 223.17 °C
Peak 235.68 °C
Endset 259.66 °C
Integral -1156.93 mJ
normalized -130.42 Jg^-1
Onset 209.26 °C
Peak 216.38 °C
Endset 221.49 °C
Integral -1077.89 mJ
normalized -121.51 Jg^-1
Onset 139.54 °C
Peak 140.73 °C
Endset 145.77 °C
Method: SAIFDS151014A
30.0-300.0°C 10.00°C/min
Lab: METTLER
STARe SW 8.10


^exo
Bromelain
Sample: SAIFDS151014A-06(B), 8.6440 mg
Integral -1701.22 mJ
normalized -196.81 Jg^-1
Onset 205.09 °C
Peak 223.40 °C
Endset 248.74 °C
Integral -1558.24 mJ
normalized -180.27 Jg^-1
Onset 73.07 °C
Peak 85.32 °C
Endset 92.20 °C
Integral -1378.77 mJ
normalized -159.51 Jg^-1
Onset 149.87 °C
Peak 154.96 °C
Endset 161.44 °C
Method: SAIFDS151014A
30.0-300.0°C 10.00°C/min
Lab: METTLER
STARe SW 8.10


^exo
Chitosan
Sample: SAIFDS151014A-05(C), 5.4610 mg
Integral -1286.94 mJ
normalized -235.66 Jg^-1
Onset 49.00 °C
Peak 89.79 °C
Endset 127.60 °C
Method: SAIFDS151014A
30.0-300.0°C 10.00°C/min
Lab: METTLER
STARe SW 8.10





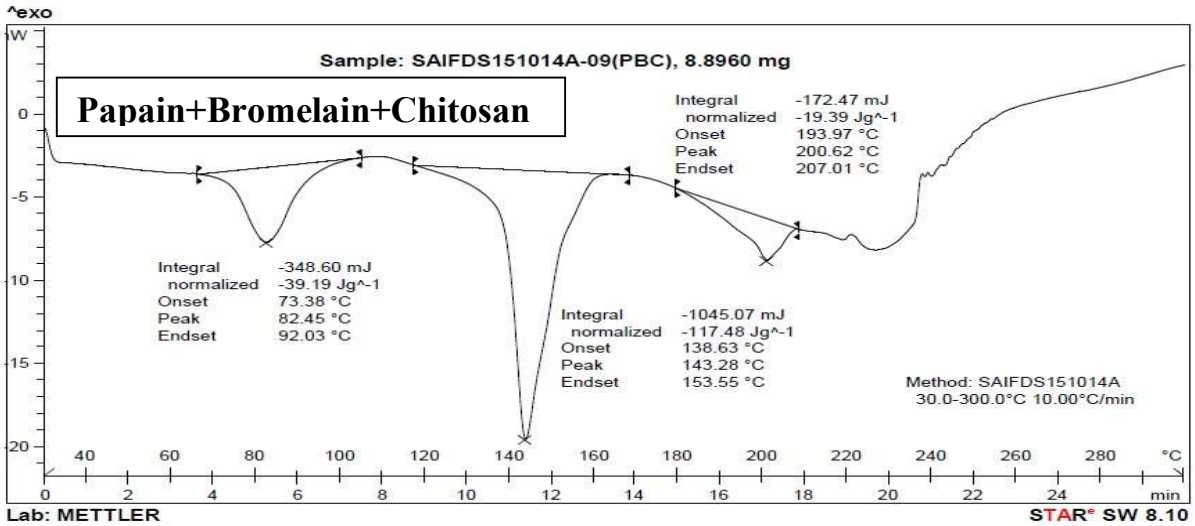


Fig.1 DSC thermogram of Papain, Bromelain and Chitosan (individual and combination)

Table-1: Formulation of topical gel

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Chitosan (g)	1	2	3	1	2	3	1	2	3	1	2	3
Na CMC (g)	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Glycerin (ml)	2	2	2	2	2	2	2	2	2	2	2	2
Methyl Paraben (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Papain (g)	-	-	-	1	1	1	-	-	-	-	-	-
Bromelain (g)	-	-	-	-	-	-	1	1	1	-	-	-
Papain + Bromelain (g)	-	-	-	-	-	-	-	-	-	1	1	1
Triethanolamine (ml)	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.
Distilled water (ml)	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.

Evaluation of prepared formulation

a) Appearance/clarity

The topical gel formulations were observed carefully by naked eye for appearance/clarity, colour, odour and presence of suspended particulate matter if any. It was further assessed by observing them against a dark and white background.

b) Determination of pH

The pH of various gel formulations was determined by using digital pH meter. One gram of gel was dissolved in 10 ml distilled water. The values were recorded immediately after preparation and after storage for 24hrs. at room temperature.

c) Determination of Viscosity

The measurement of viscosity of the prepared gel was done with a Brookfield Viscometer. The gels were rotated at 100 rotations per minute. The viscosity values were noted [12]

Test conditions

- Type of equipment-Brookfield RVDV-II +Pro
- Spindle- T-bar
- Spindle code –S 96
- Sample volume- 10.0ml
- Rpm –100

d) Spreadability

It indicates the extent of area to which gel readily spreads on application to skin or affected part. The therapeutic potency of a formulation also depends upon its spreading value. It is expressed in terms of time in seconds taken by two slides to slip off from gel. 1gm gel is placed in between the slides under the direction of certain load. Lesser the time taken for the separation of two slides, better the Spreadability [13].

It was calculated using the following formula:

$$S = M\ x\ L/T$$

Where, S – Spreadibility
M – Weight in the pan
L – Length moved by the glass slide
T – Time (in sec.) taken to separate slides completely.

e) Bioadhesive strength

For this, Individual samples of gel formulation were applied to the base of inverted glass vial using double sided adhesive tape to secure the gel in position. The distance between two vials was adjusted in such a way that the gel sample remain adhere to mucosal membrane.

Sufficient pressure was applied on both side of the vial for 10 sec to allow proper adhesion of the gel to mucosa. A constant weight was added to the pan connected to the other arm of modified balance which pulls the vial away from the other vial. The weight required for detaching the two vials was noted. The bioadhesive force expressed as the detachment stress in dynes/cm^2 was determined from the minimum weight that detached the mucosal tissue from surface of each formulation.

Bioadhesive strength (dynes/cm^2) = Mg /A

Where, M= weight required for detachment in gram,
g = acceleration due to gravity (980 cm/s^2)
A = area of mucosa exposed

f) Extrudability

It was used for determination extrudability of gel. A collapsible tube filled with a gel, then pressed firmly at the crime end. When the cap was removed, gel extrudes until pressure dissipated. Weight in grams required to 0.5cm ribbon of gel was determined in 10 sec. Average extrudation pressure in gram observed.

g) Skin irritation test

It was performed on healthy human volunteers. 5 volunteers were selected for each gel and 1 g of formulated gel was applied on an area of 2 sq mm. to the back of hand. The volunteers were observed for lesions and irritation.

h) Accelerated stability studies

All the selected formulations were subjected to a stability testing three months as per ICH guidelines at a temperature of 40 ± 2^{0} and were analyzed for the change in appearance, pH and drug content [14].

RESULT AND DISCUSSION

Investigation of physicochemical compatibility of plant enzymes and polymer

The DSC thermograms of papain, bromolein and chitosan are shown in Fig.1 . The pure enzymes (Papain and bromelain) showed a very pointed endothermic peak at (216.38^{o}C and 154.96 ^{o}C) with peak onset at (139.54 ^{o}C

and 149.87 ^{o}C) respectively, which corresponds to its melting point. The sharp endothermic peak of both the enzymes confirms crystallinity in structure. Whereas, chitosan exhibits broad endothermic peak (89.79 ^{o}C) over the entire scanning range of 30°C to 300°C, suggesting its amorphous nature. However, no characteristic peak of enzymes was found in formulation over the tested temperature, which clearly indicated that the drug is in amorphous state or high-energy state after the fabrication and interacted with the polymer. Since amorphous state is considered as a state of high disorder, the solid drugs present remain in highly dissolved state within the polymer matrix in the formulation.

Evaluation of prepared formulation

The topical gels were prepared and subjected to physical evaluations such as appearance, pH, viscosity, spreadability, bioadhesive strength, extrudability and skin irritation (Table-2). The gels were clear throughout the evaluation. The pH was constant (6.8 – 7.0) and did not produce irritation upon application to the skin. Viscosity, Bioadhesive strength and Extrudability was excellent while spreadability was less variant. Furthermore, the stability studies results of selected formulation revealed that formulation was stable under normal storage condition (Table-3).

Table-2: Physical evaluation of the prepared Formulation

Formulation Code	Appearance	pH	Viscosity (cps x 10^3)	Spreadability (g.cm/sec)	Bioadhesive strength (gm/cm^2)	Extrudability (gm /cm^2)	Skin irritation
F1	Clear	6.8	79	15.65	1.10	17.00	nil
F2	Clear	6.8	82	13.45	1.25	15.50	nil
F3	Clear	6.9	85	12.36	1.32	14.53	nil
F4	Clear	6.8	81	14.60	1.15	16.65	nil
F5	Clear	7.0	84	13.00	1.22	15.05	nil
F6	Clear	6.8	87	11.40	1.45	13.35	nil
F7	Clear	6.9	80	15.20	1.00	17.30	nil
F8	Clear	6.8	84	13.30	1.20	15.40	nil
F9	Clear	6.8	86	11.70	1.32	13.80	nil
F10	Clear	6.9	85	12.40	1.28	14.60	nil
F11	Clear	6.8	88	11.05	1.42	13.10	nil
F12	Clear	7.1	92	10.20	1.55	12.25	nil

Table-3: Accelerated stability studies

Sr. No.	Batches	Months	Appearance	pH	Drug Content (%)
1	F2	0	Clear	6.8	99.75
		1	Clear	6.8	98.50
		2	Clear	6.9	97.30
		3	Clear	6.9	96.00
2	F5	0	Clear	7.0	99.85
		1	Clear	7.0	98.70
		2	Clear	6.9	97.20

		3	**Clear**	**6.9**	**96.10**
3	**F8**	**0**	**Clear**	**6.8**	**99.90**
		1	**Clear**	**6.9**	**98.40**
		2	**Clear**	**6.9**	**97.10**
		3	**Clear**	**6.9**	**96.15**
4	**F11**	**0**	**Clear**	**6.8**	**99.90**
		1	**Clear**	**6.8**	**98.60**
		2	**Clear**	**6.7**	**97.30**
		3	**Clear**	**6.8**	**96.10**

CONCLUSION

Herbal formulations have growing demand in the world market as natural products are more acceptable in the belief that they are safer than synthetic one. It was good attempt to prepare a herbal topical gel containing plant enzyme. The results of above study were promising. Since, the topical gel easily washable and has wider prospects to be used in skin disorders.

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