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HERBALLY MEDICATED HYDROGEL FOR DIABETIC FOOT ULCER

Swathi P. Nair* and Gowtham M.

Department of Pharmaceutics, Rajiv Gandhi Institute of Pharmacy Trikaripur (P.O) Kasaragod, Kerala, 671310.

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***Corresponding Author**
Swathi P. Nair
Department of Pharmaceutics, Rajiv Gandhi Institute of Pharmacy Trikaripur (P.O) Kasaragod, Kerala, 671310.

ABSTRACT

India, the land of Vedas, has always stood in its prime position in terms of culture and tradition. Indians have formulated several medicine, right from the ancient time, which are still used as remedies for several aliments. Herbal medicines not only cure the disease; but heals the body as whole. The sagas in ancient India, developed these magic remedies, without the aid of technological advances. Herbal medicine have better patient compliance because of their long term curing capacity and absence of side effects, The present study has been undertaken with the aim of formulate and evaluate the diabetic wound healing effects of polyherbal hydrogels prepared from ethanolic extracts *Catharanthus roseus and Terminalia chebula* followed by *Aloe barbadensis* gel. Phytochemical screening showed that alkaloid, triterpenoid, tannin, phenols are responsible for the activity. The hydrogel was formulated using different polymer (Carbapol 934, HPMC K 100, Chitosan) at a concentration of 1.5%w/w. Concentration of plant extract remain constant (2:4:1). Physical parameters like pH, spredablity, viscosity, swelling index were evaluated and they showed better result. pH of the formulation was compatable with skin pH. The gel formulation F2 containing carbapol polymer was selected as the optimised formulation. All the formulated hydrogel were subjected to Antioxidant studies F2 showed lowest IC_{50} values. F2 was subjected to *in vivo* evaluation. Steptozotocin was used to induce diabetic on rats. Excision wound model was used to evaluate diabetic wound healing. Percentage wound contraction was compared with standard Mega heal ointment. Stability studies revealed that the formulated hydrogel was stable at room as well as refrigerating temperature.

KEYWORDS: Herbal medicine; hydrogel; Diabetic wound healing; *Catharanthus roseus*; *Terminalia chebula; Aloe barbadensis.*

INTRODUCTION

Herbal medicine, also called as home grown cures, phytotherapeutic specialists herbal remedies, phytopharmaceuticals and so on, are the most established and still the most broadly utilized arrangement of medication on the planet today. Ever since the birth of mankind there has been a relationship between life, disease and plants. Home grown cures were exchanged to age as people medication. So the historical backdrop of home grown medication is as old as mankind's history.[1] It was utilized by man since old times as the main line treatment for a lion's share of ailments. Because of the availability, lesser side effects and lesser reactions, natural medications still thrive and are finding wonderful acknowledgment in both the creating and the created nations. Therapeutic plant have particular properties and particular utilization owing to the organic gathering of mixes and has an extraordinary significance in person as it demonstrates assorted pharmacological properties. Phytochemicals were utilized by man for the treatment of different illnesses, for example, cardiovascular scatters, cancer-causing inconveniences, metabolic disarranges, urinary disorders etc.[2] The Indians have known about the magical properties of herbs and advocated their use in many of the ancient scripture. In the mid twentieth century natural prescriptions were prime human services framework, as anti-infective agents or analgesics were not accessible. With increasing utilization of allopathic arrangement of medication, natural prescription step lost its notoriety among individuals and it depended on the quick restorative activities of manufactured synthetic medications. Centuries has passed and allopathic system became the ruler of disease management. Recently herbal pharmaceuticals won the new era because people recognised the herbal preparation can be more effective than conventional medicines and their non-toxic nature means that they can be administrated over long period**.**

Beings Diabetes, the patient has 25% danger of building up a foot ulcer amid his lifetime, half of which get contaminated, infected and if untreated, brings amputation of legs. Consistently, right around 83,000 lower appendage removals are performed because of the DFUs, additionally connected with mortality. As the number of diabetic patients are increasing day by day in the same range rate of foot ulcer is also increasing.[3] Now a days foot complications of diabetes are quiet common. Wound might be created by physical, thermal, chemical, microbial or immunological injury to the tissue. Diabetic foot wounds are

characterized as any break in the cutaneous barrier, normally stretching out through the full thickness of the dermis.[4] There have been diverse techniques for contamination control and treatment of diabetic foot ulcers.

Elevated blood glucose levels for longer duration affects the veins and it causes decreased blood steam to the foot. The cells present in the foot could not get sufficient oxygen and nutrients. The high blood glucose level causes loss of sensations so that the patient having elevated blood glucose levels could not recognise any pain or any wound on the legs. Loss of sensation, nerve damage and high blood glucose increase the complication of foot ulcers. Their principle objective is to quicken the injury mending and tissue repair. The process of wound repairing consists of coordinated cell and biochemical events prompting restoration of stucture and functional integrity with recapture of strength of the harmed tissue. Limb amputation is the last and final option if the wound is not properly healed. Highly efficient and success treatment plan for foot ulcer is patient education.[5,6]

Catharanthus roseus is an evergreens shrub of the family apocynaceae. There are 8 species[7] of catheranthus, 7 species Are endemic to Madagascar and one is endemic to india (c.pusillus). *Catheranthus roseus* have long history,The plant was traced by Mesopotominanns at 2600 bc. Based on the colour there are 2 main species *Cartharanthus roseus* having pink and *Catharathus alba*[8] having white colour. *C Roseus* is the rich source of alkaloid so that the plant plays key role in world health care. A large number of indole alkaloids are present in vinca. Out of them about 20 dimeric indole dihydroindole alkaloid posesesses oncolytic activity, and among them, vincristine and vinblastine are most significant. Vinblastin contains indole alkaloid part called catharanthine and dihydroindole alkaloid part vindoline.The other alkaloid present in vinca is ajmaline, lochnerine,serpentine, and tetrahydroalstonine. It requires about 500mg crude drug to extract out 1 g of vincristine, Because of its extreame low content i.e. 0.0002 per cent. These alkaloids very are costlier.[9,10]

Terminalia chebula is a flowering deciduous tree of the family combrataceae. According to hindu mythology the plant is considered as it is originated from the drops of amrita so it is called king of medicines. The Terminalia consists of 250 species and widely distributed in tropical areas of the world[11] *Terminalia chebula* contain several phytochemicals such as tannins fructose amino acids, resins flavonoids, sterols, but the major constituent is tannin approximately 32 percentage, the tannin contents varies according to the geographical

locations. Phytochemicals like aminocaids fructose resins fixed oil, carbohydrates etc. are also present in this plant. *Terminalia chebula* is a rich source of hydrolysable tannin like like gallic acid, chebulic acid, punicalagin, chebulanin, corilagin, neochebulinic, ellagic acid, chebulagic acid, 1,2,3,4,6 -penta-orgalloyl-β-D-glucose 1,6-di-o-galloyl-D-glucose and terchebulin. These are responcible for pharmacological activities. Phytochemicals like anthraquinone ethadioic acid, sennoside,4,2,4-chebylyl-d-glycopyranose terpenes and terpinols have also been reported Some other minor chemical constituents were polyphenols such as corilagin, gallolyl glucose, punicalagin,terflavinA, maslimic acid. Fructose, amino acid, succinic acid, b etasitosterol. The present work was aimed to a comparative study on different polymers for the preparation of hydrogel containing ethanolic extracts of *Catharanthus roseus, Terminalia chebula* and fresh aloe vera gel.[12,13]

MATERIALS AND METHODS

Collection of plants

Fresh leaves of Catharanthus roseus and fruit of Terminalia chebula and fresh Aloe barbadensis were collected from Kasargod district, Kerala, India in the month of October 2016 were authenticated by Dr. A Rajagopalan Professor, Dept. of Horticulture, Padannakad, Kasaragod, Kerala.

Determination of physicochemical paramaters

The collected plant materials were washed properly under running tap water followed by sterilized distilled water and were completely air dried at room temperature. The dried plant materials then coarsely powdered in an electronic blender and stored in air tight containers until further use. Physico-chemical investigation of formulations were carried out for the determination of total ash, acid insoluble ash, water soluble ash, moisture content by loss on drying method, water and alcohol soluble extractive values.

Preparation of plant extract[14]

The collected plant materials for the study were first washed with running tap water followed by sterilised distilled water and completely air dried at room temperature, then it was coarsely powdered using electronic blender and stored in an airtight containers. Aloe vera gel was collected from fresh aloe plant. Maceration is the process selected for the plant extract preparation. For that 10gm of dried coarsely powdered drug were taken in different beaker with 100 ml of solvent such as petroleum ether, chloroform, ethanol, water. Then the beaker was allowed to stand on awater bath for 30m with occasional shaking. Finally each extract

were fillterd through Whatmann filter paper grade 1 and concentrated, labelled and stored in a refrigarator for further use to detect the phytochemicals present in the different extracts.

Preliminary phytochemical analysis[15, 16]

Preliminary qualitative analysis of all the extracts (petroleum ether, chloroform, ethanol and water) was carried out by employing standard conventional procedures

Soxhlet extraction of plant material[17]

Soxhlet extraction is a continuous solid/ liquid extraction. 2.5 Kg of plant material for the study was shade dried and coarsely powdered. Both the drugs are extracted with 95% of ethanol. The process lasted until the solvent present in siphon tube becomes colourless. Ethanol in the extract was recovered by distillation process. Air dried and concentrated. The extract obtained was weighed and the percentage yield was calculated. Aloe vera gel is collected from fresh aloe vera plant.

Drug excipient compatability study

The FT-IR spectrum of drug extracts with other ingredients was analyzed for compatibility study.

Formulation of hydrogel

Same combination of Catharanthus roseus, Terminalia chebula and aloe barbadenisis extract were treated with different polymer such as carbapol 934, HPMC, Chitosan using various formulae.

Table no. 1: Composition of polyherbal hydrogel.

Ingredients	Formulation code									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Catharanthus roseus leaves extract (mg)	200	200	200	200	200	200	200	200	200	200
Terminalia chebula fruit extract (mg)	100	100		100		100		100		100
Aloe vera extract(mg)	50	50	50	50	50	50	50	50	50	50
Carbapol 934	1.5	-	-	1	1	-	0.5	0.5	-	0.5
HPMC K 100	-	1.5		0.5	-	1	1	-	0.5	0.5
Chitosan	-	-	1.5	-	0.5	0.5		1	1	0.5
Propylene glycol (ml)	5	5	5	5	5	5	5	5	5	5
Ethanol(ml)	3	3	3	3	3	3	3	3	3	3
Dispdium EDTA(g)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Methyl paraben (g)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben (g)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Tri ethanolamine	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Distilled water	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml

Procedure[18]

Total amount of water required for the formulation was divided in to two, large volume portion and small volume portion. Weighed quantity of polymer was slowly sprinkled on the large portion of water with vigorous shakeing using mechanical stirrer. Extract was weighed and dissolved in ethanol to this add propylene glycol.then take the small volume portion of water and required quantity of methyl paraben and propyl paraben were dissolved by heating in a water bath and disodium EDTA was added. At last all the ingredient were mix properly with polymeric solution and make up the volume made up by adding the distilled water. Then triethanolamine was added drop wise with continuous stirring to adjust the skin pH and consistency.

Evaluation

Physical evaluation

Physical parameter such as colour, appearance were evaluated.

Homogenity

All the developed formulation were tested for homogeneity by visual appearance.

pH

pH of various gel formulation were determined by using digital pH meter. 2.5g of gel was accurately weighed and dispersed in 25 ml of distilled water and stored for two hours. The measurement of pH of each formulation were carried out in triplicate and average value was calculated.[19]

Spredability

Test formulations 1 g each were placed with in a circle of 1 cm diameter pre marked on a glass plate over which a second glass plate was placed. A weight of 5 g was allowed to rest on the upper glass plate for 5 min. the increase in the diameter due to spreadability of the formulation was noted.[20]

Viscosity

The viscosity of the gel was measured by using Brookfield viscometer. For this 200g gel was taken in a beaker and spindle was diped in the beaker for 5 minutes at 25^0C, and the reading was noted.[21]

Swelling index[22]

To determine the swelling index of prepared topical gel, 1 gm of gel was taken on porous aluminum foil and then placed separately in a 50 ml beaker containing 10 ml 0.1N NaOH. Then samples were removed from beakers at different time intervals and put it on dry place for some time after it reweighed. Swelling index was calculated as follows

Swelling index = (wt- wo)/wt *100.

Extrudability[23]

All gel formulation were filled in standered capped collapsible aluminium tubes and it is sealed. The weight of the tubed were recorded and the tubes were placed between two glass slide and were clamped. 500gm weight was placed over slides and the cap was removed. The amount of excruded hydrogel was collected and weighed. The percentage of extruded gel was calculated as.

1. When it is greater than 90% then extrudability is excellent
2. When it is greater than 80% then extrudabilty is good
3. When it is 70% then extrudability is fair

In vitro Antioxident study[24]

A solution of hydrogen peroxide (20 mM) was prepared in PBS (pH 7.4). Various concentrations of 1 ml of the samples or standards in methanol were added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min against blank solution that contained extracts in PBS without hydrogen peroxide. IC50 value is the concentration of the sample required to scavenge 50% free radical. The above experiments were performed (in triplicate) and the percentage inhibition was calculated using the following formula: Where A0 was the absorbance of the standard (ascorbic acid) and A1 was the absorbance of samples.

%scavenged [H2O2]=[(A0−A1)/A0]×100,

Invivo study

Invivo studies are performed after the approval of institutional animal ethics committee(1AEC).

Skin irritation study[22]

0.5 gms of the herbal gel was used as the test substance was applied to an area of approximately 6 cm^2 of skin and covered with a gauze patch. The patch was loosely held in contact with the skin by means of a semi-occlusive dressing for the duration of 1 hour and gauze was removed. At the end of the exposure period, i.e, 1 hour, residual test substance was removed, Observations have recorded after removal of the patch. Control animals were prepared in the same manner and 0.5 gms of the gel base i.e., gel formulated using all ingredients except the herbal mixture was applied to the control animals and observations were made as similar to the test animals. The gel was applied to the skin once a day for 7 days and observed for any sensivity and the reaction if any was graded as : A – No reaction, B – Slight patchy erythema, C – Slight but confluent or moderate but patchy erythema, D – Moderate erythema, E – Severe erythema with or without edema.

Streptozotocin-nicotinamide induced excision wound model.[25,26]

For the study 24 healthy albino wister rats weighing 200 -3000g were used. The animals were kept individually in polypropylene cages having paddy husk as bedding. Diabetes was induced by giving single dose intraperitonial injection of steptozotocin (45mg/kg) after the administration of 110 mg/kg nicotinamide in o. 1 M citrate buffer and normaline saline respectively.

For next I day rat were given glucose solution to prevent from death. After 72 hour the fasting blood glucose level was measured.The rat showing fasting glucose level 250mg/dl selected for the study.

At the begning the rats are anasthaized and the right hind foot was taken and an excision wound of size 1cm*1cm was made by cutting the skin of the foot.

The rats are givided into 4 of six each

Group1: Normal control

Group 2: Standard control

Group 3 Diabetic foot ulcer wih mega heal gel

Group 4: diabetic foot ulcer with polyherbal preparl

The wound was trated with herbal and standard gel for 11 day and the percentage of wound contraction was measured on first third seventh and eleventh day using a transparent paper and permenant marker.

Decrease in the Wound size was calculated using the formula.

$$\textbf{Wound contraction (\%)} = \frac{wo\text{-}wt}{Wo} * 100$$

Statistical analysis

The results were expressed as mean ± Standard Deviation (SD). The data were subjected to one-way ANOVA followed by Dunnet's test. The differences were considered as statistically significant at $P<0.05$, when compared with control.

Stability study

Study was performed as per ICH guidelines. The formulated gel were filled in collapsible tubes and stored at different temperatures and humidity conditions like ambient temperature (R.T), refrigerator temperature (8±10C) and condition of accelerated stability testing (450C±20C/ 75% ±5% RH) for a period of three months and studied for appearance, pH and spreadability.

RESULT AND DISCUSSION

Plant collection and authetification

The plants *Catharanthus roseus*, *Terminalia chebula*, and *Aloe barbadensis* were collected from Kasargod and Kannur district, Kerala in the month of November and December 2016 and were authenticated by Dr. A Rajagopalan, professor, Dept. of Horticulture, Padannakad, kerala.

5.2. Physico-chemical parameters.

After the collection of plant material the plants were washed with running water and shadow dried and powdered coarsely and stored in air tight containers. Physico chemical parameter such ash value, extractive value, moisture content were evaluated and reported.

Table No 2: physico- chemical parameter of *T. chebula, C. roseus, A.barbadensis.*

Sl. No	Test	*Terminalia chebula*	*Catharanthus roseus*	*Aloe barbadensis*
1	Total ash(%w/w)	6.73±0.05	0.35.±0.03	1.71±0.05
2	Acid insoluble Ash(%w/w)	0.43±0.02	0.56±0.03	9.22±0.03
3	Water souble Ash(%w/w)	5.84±0.03	1.4±0.01	37.56±0.02
4	Water soluble extractive value(%w/w)	66.38±0.17	7.41±0.04	17.23±0.09
5	Alchohol soluble extractive value(%w/w)	51.38±0.16	3.36±0.09	8.47±0.02
6	Moisture content (%w/w)	7.34±0.08	9.71±0.05	11.47±0.02

Value are expressed in mean ± SD,(n=3)

Preliminary phytochemical screening

Table No. 3: Phytochemicals present in aqueous, ethanol, petroleum ether and chloroform extract of *Catharanthus roseus.*

Compounds	Petroleum ether	Chloroform	Ethanol	Aqueous
Alkaloid	+	+	++	+
Phenols	++	+	++	++
Carbohydrate	-	+	+	+
Flavanoid	+	-	+	-
Tannin	++	+	+++	++
Aminoacid	-	-	-	-
Terpenoid	-	+	++	+
Saponin	-	-	+	+

(+++)- Intensively present, (++)- Moderately present, (+)- Present, (-)- Absent.

Table No. 4: Phytochemicals present in aqueous, ethanol, petroleum ether and chloroform extract of *Terminalia chebula.*

Compounds	Petroleum ether	Chloroform	Ethanol	Aqueous
Alkaloid	+	+	+	-
Phenols	+	++	++	+
Carbohydrate	+	+	+	+
Flavanoid	+	+	+	+
Tannin	++	+	+++	++
Aminoacid	-	-	-	-
Terpenoid	-	-	-	-
Saponin	-	-	-	-

(+++)- Intensively present, (++)- Moderately present, (+)- Present, (-)- Absent

Table No. 5: Phytochemicals present in aqueous, ethanol petroleum ether chloroform extract of *Aloe barbadensis.*

Compounds	Petroleum ether	Chloroform	Ethanol	Aqueous
Alkaloid	+	+	+	+
Phenols	-	+	+	+
Carbohydrate	+	+	+	+
Flavanoid	+	-	+	+
Tannin	+	+	+++	-
Aminoacid	+	+	+	+
Terpenoid	+	-	-	-
Saponin	-	+	+	-

(+++)- Intensively present, (++)- Moderately present, (+)- Present, (-)- Absent

The phytochemical screening on various extract of *Terminalia chebula, Catharanthus roseus, Aloe barbadensis* showed the presence of various phytochemical such as alkaloid, tannin, flavonoid and phenols. Compared to other solvent ethanolic extract shows abundant presence of phytoconstituent, so ethanol is taken as a menstrum for further extraction

Soxhlet extraction of plant material

The dried coarsely powdered leaves of *Catharanthus roseus* and fruit of *Terminalia chebula* were extracted by continuous soxhlet extraction using ethanol as menstrum. The extract obtained from the plants was collected and concentrated. Which was then weighed and kept in a desiccator containing calcium chloride until it was used for the further studies. Aloe vera was collected from fresh plant leaves. The yield obtained was shown in table.

Table No 6: Percentage yield of ethanolic extract of *C.roseus* and *T. chebula.*

SL.NO	Sample	Amount of sample taken (g)	Amount of extract obtained (g)	% yield
1	*Catharanthus roseus*	50	7.9	15.8
2	*Terminalia chebula*	50	9.2	18.4

Table No 7: The % yield of Aloe gel from fresh *Aloe barbadensis* plant

SL.NO	Sample	Amount of sample taken (g)	Amount of extract obtained (g)	% yield
1	*Aloe barbadensis*	10	8.73	87.3

Drug excipient compatability study

The compatability test is used to check the compatibility between the drug and excipient used in the formulation. The FT-IR spectrum were obtained using JASCO FTIR 4700L spectrophotometer.

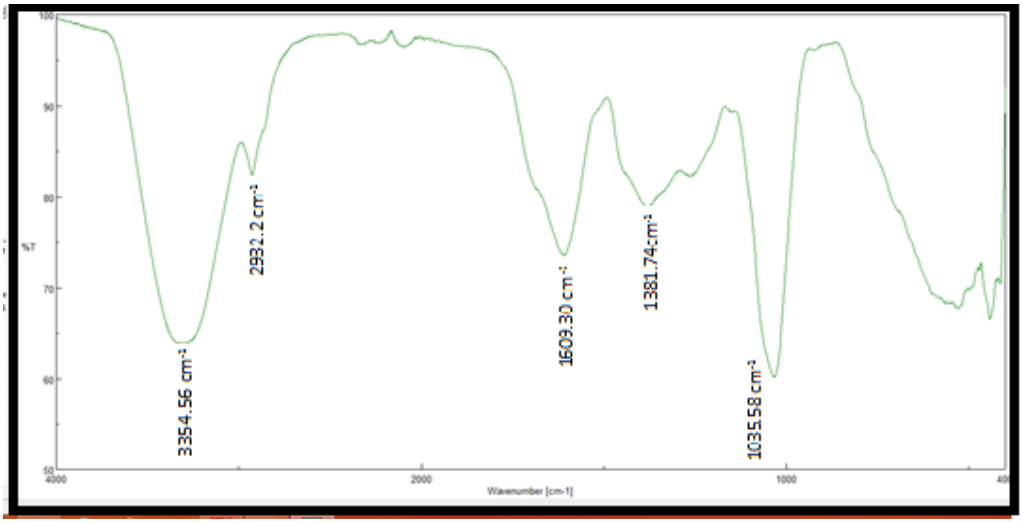


Figure 1: FT-IR spectum of *Catharanthus roseus.*

Sample A: 3354 cm^{-1} (O-H streching), 2932.2 cm^{-1} (C-H streching), 1609.30 cm^{-1}(N-H strching), 1381.74 cm^{-1} (C-H_3 bending), 1035.58 cm^{-1} (C-N streching).

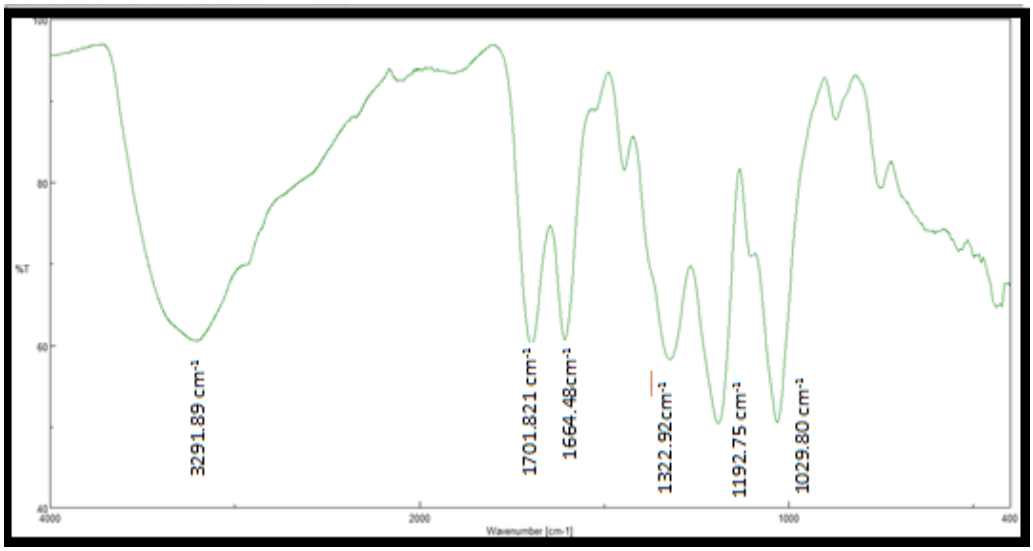


Figure 2: FT-IR spectrum Terminalia chebula.

Sample B: 3291.89 cm^{-1} (O-H streaching), : 1701.61 cm^{-1}(N-H bending), 1664.44 cm^{-1}(N-H bending), 1322.92 cm^{-1}(C=C streaching) 1192.75 cm^{-1}(C-N stretching), 1029.80 cm^{-1}(C-N stretching).

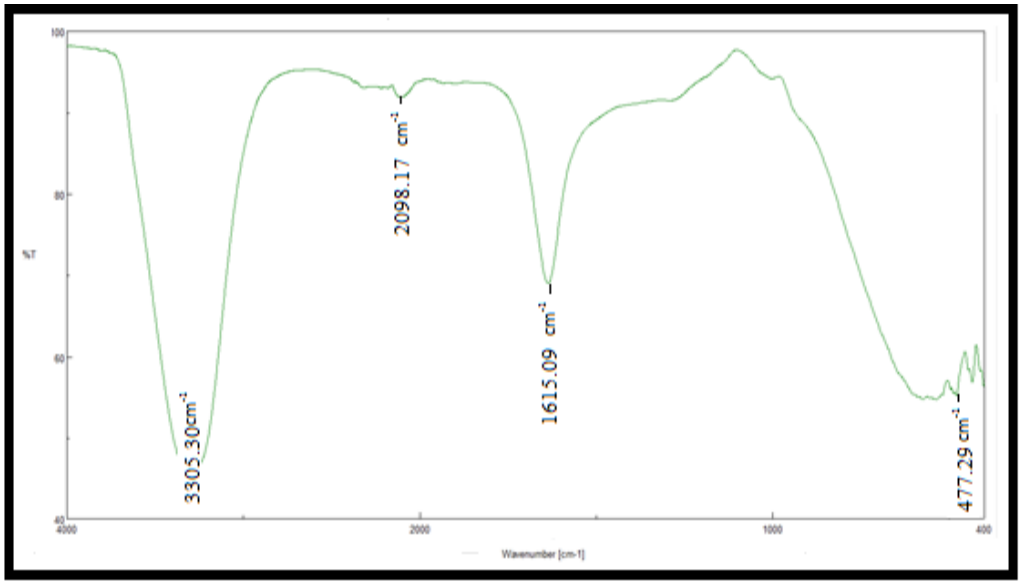


Figure 3: FT-IR spectrum of *Aloe barbadensis.*

Sample C: 3305.39cm^{-1}(Alcohol/phenol, O-H stretch), 1615.09 cm^{-1} (N-H bending), 477.29 cm^{-1} (C-I stretching).

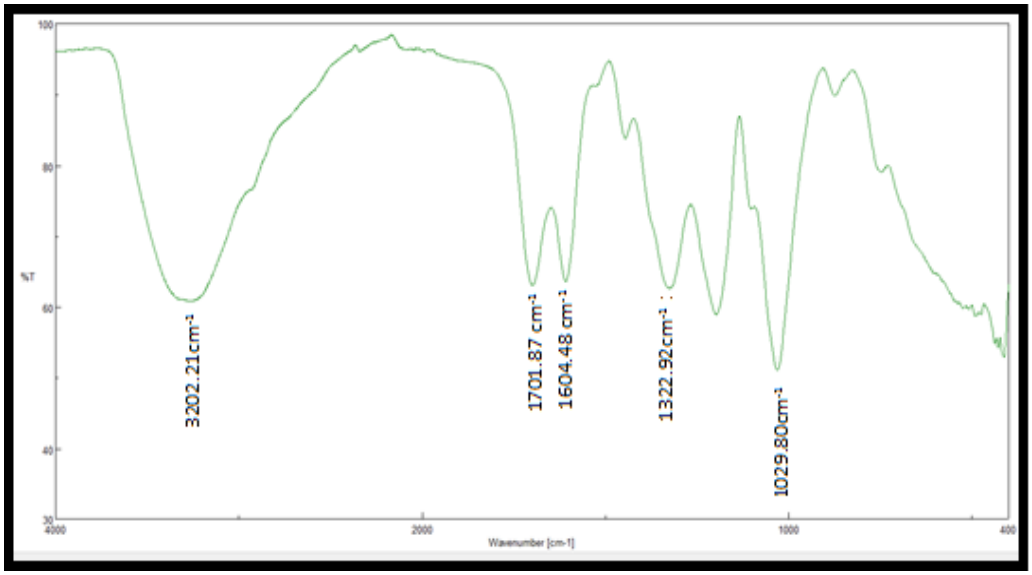


Figure 4: FT-IR spectrum of Mixture of *Catharanthus roseus, Terminalia chebula and Aloe barbadensis.*

Sample D: 3202.21cm^{-1} (O-H stretching), 1701.87 cm^{-1} (N-H bending) 1604.48 cm^{-1} (N-H bending) 1322.92cm^{-1}(C-H_3 bending) 1029.80cm^{-1}(C-N stretching).

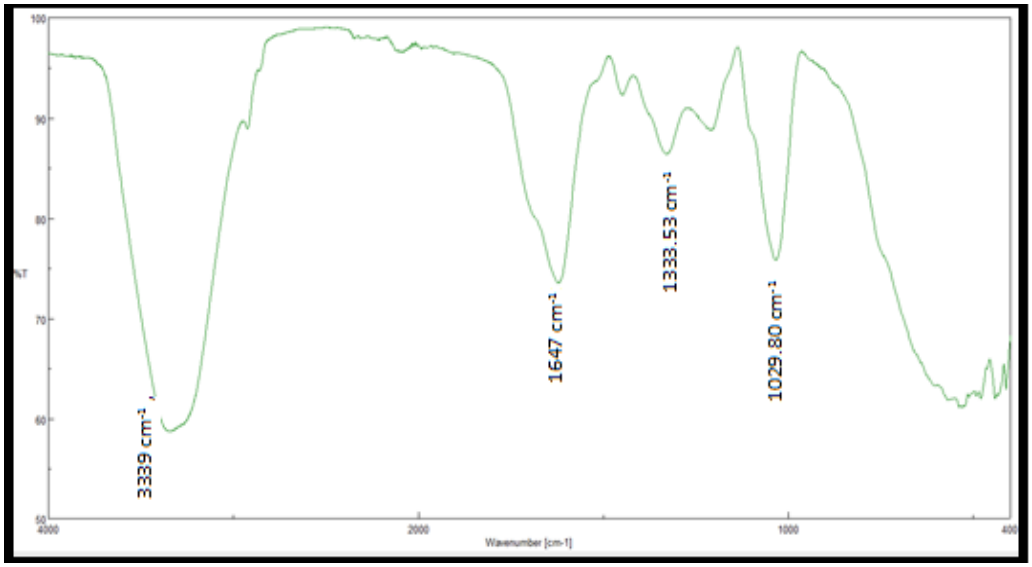


Figure 17 : FT-1R spectrum of mixture of drug with HPMC.

Sample E: 3339 cm^{-1} (O-H stretching), 1647 cm^{-1}(N-H bending), 1333.53 cm^{-1} (C-H_3 bending), 1029.80 cm^{-1}(C-N stretching).

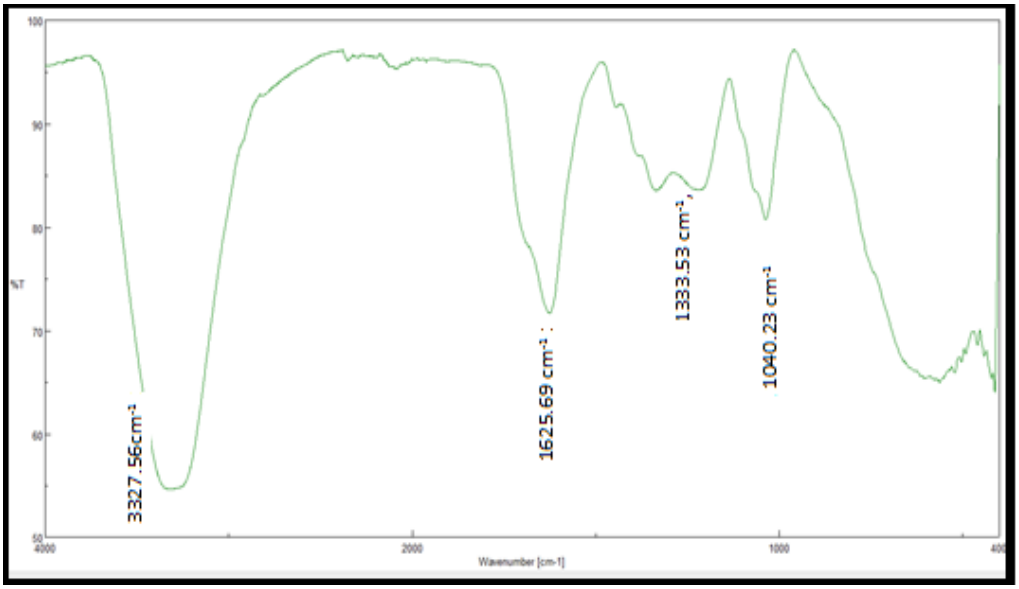


Figure 5: FT-1R spectrum of mixture of drug with Carbapol 934.

Sample F: 3327.56cm^{-1}(O-H stretching), 1625.69 cm^{-1} (N-H bending), 1333.53 cm^{-1}(C-H_3 bending), 1040.23 cm^{-1}(C-N stretching)

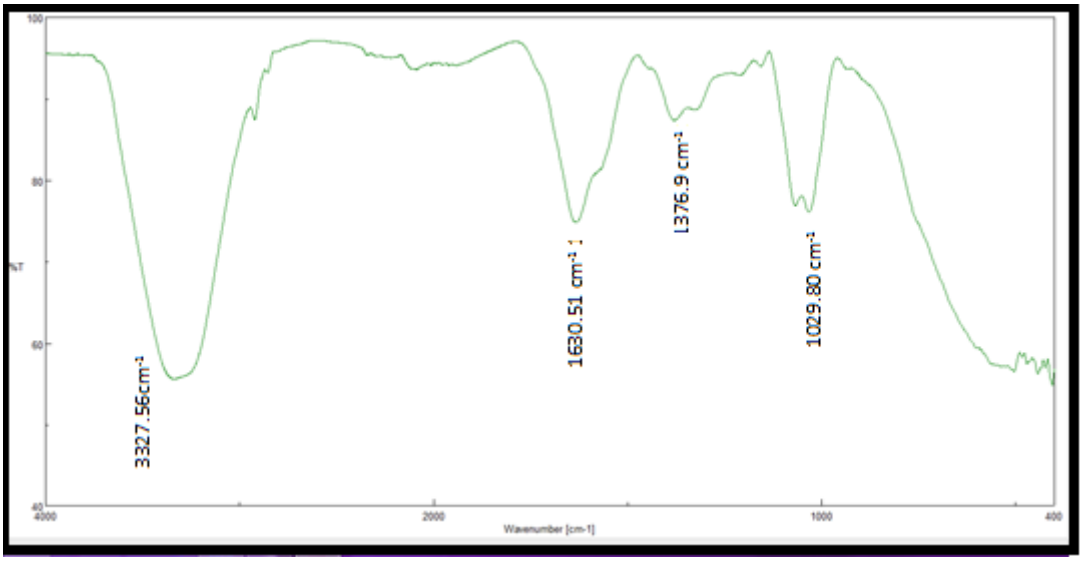


Figure 6: FT-1R spectrum of mixture of drug with Chitosan.

Sample G: 3327.56 cm^{-1} (O-H stretching), 1630.51 cm^{-1}(N-H bending), 1376.9 cm^{-1} (C-H_3 bending), 1029.80 cm^{-1}(C-N stretching).

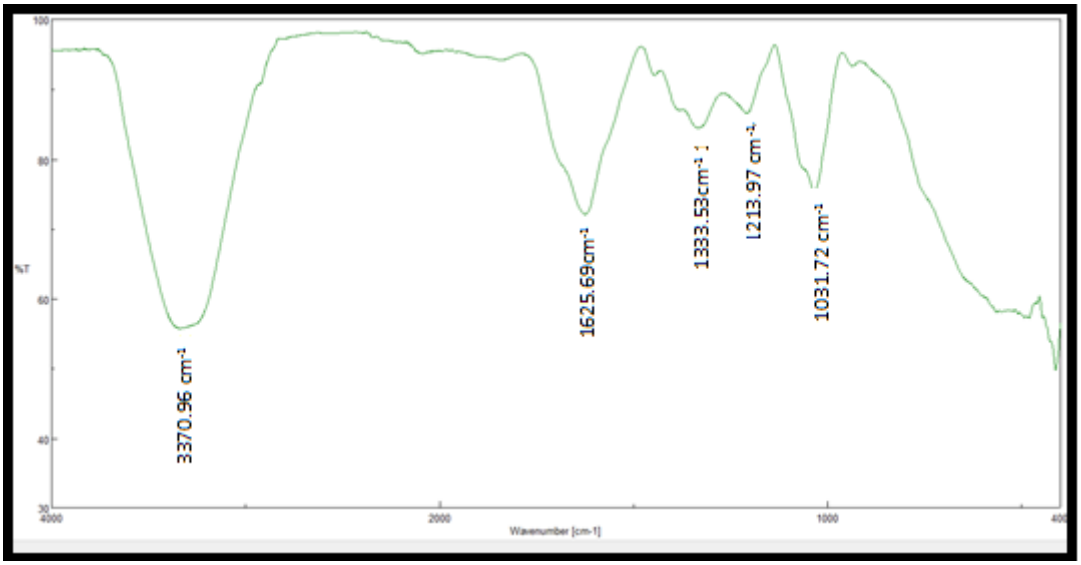


Figure 7: FT-1R spectrum of mixture of drug with Mixture of polymer.

Sample H :3370.96 cm^{-1} (O-H stretching), 1625.69 cm^{-1}(N-H bending), 1333.53 cm^{-1} (C-H_3 bending), 1213.97 cm^{-1} (C-H_3 bending), 1031.72 cm^{-1}(C-N stretching).

Formulation of hydrogel

10 formulation named F1-F10 were formulated using single and combination of different polymers such as carbapol 934, HPMC and Chitosan.

Evaluations

Physical evaluation

Hydrogel was formulated using different polymers (Carbapol 934, HPMC K 100, Chitosan) were the concentration of ethanolic extract of the plant remain constant Physical parameter of hydrogel such as colour and appearance, homogeneity, washability, extrudability was evaluated. All the formulation are homogenous, yellowish brown in colour, clear translucent in nature and possess good washability. In the case of extrudability F1 and F2 showed excellent extrudability, while all other formulations possess good extrudability characters.

The *in-vitro* evaluations were performed such as pH, spreadabilty, viscosity, swelling index, extrudabilty, antioxidant studies.

Table No 8: Physical parameters of hydrogel

Sl.No	Formulation code	Colour	Apperance	Homogenity
1	F1	Yellowish brown	Clear and translucent	Homogeneous
2	F2	Yellowish brown	Clear and translucent	Homogeneous
3	F3	Yellowish brown	Clear and translucent	Homogeneous
4	F4	Yellowish brown	Clear and translucent	Homogeneous
5	F5	Yellowish brown	Clear and translucent	Homogeneous
6	F6	Yellowish brown	Clear and translucent	Homogeneous
7	F7	Yellowish brown	Clear and translucent	Homogeneous
8	F8	Yellowish brown	Clear and translucent	Homogeneous
9	F9	Yellowish brown	Clear and translucent	Homogeneous
10	F10	Yellowish brown	Clear and translucent	Homogeneous

All developed hydrogel shows yellowish brown in colour and clear translucent in appearance. Homogenity is evaluated by visual appearance all formulated hydrogel is taken in a petridish and homogeneity is evaluated by visual appearance. The result found that all hydrogel is homogeneous in nature.

Table 9: pH, spreadability, viscosity and extrudability of the formulations.

Sl.No	Formulation code	pH	Viscosity	Spredability	Washability	extrudability
1	F1	6.72±0.01	16854±2	5.16±0.03	Good	Excellent
2	F2	6.85±0.01	16933±2.5	5.52±0.01	Good	Excellent
3	F3	6.12±0.02	15935±1.52	4.24±0.02	Good	Good
4	F4	6.66±0.02	16235±3.21	4.94±0.02	Good	Good
5	F5	6.24±0.01	16625±2.51	3.9±0.02	Good	Good
6	F6	6.41±0.02	15744±1.52	4.75±0.03	Good	Good
7	F7	6.34±0.01	16566±2.51	4.34±0.03	Good	Good

8	F8	6.2±0.02	16115±2.64	3.34±0.02	Good	Good
9	F9	6.46±0.01	15437±1.5	4.53±0.02	Good	Good
10	F10	6.28±0.01	15876±2.51	5.23±0.02	Good	Good

The pH of all formulation F1 –F10 was measured using digital pH meter. The formula shows pH in the range of (6.12±0.02) to (6.85±0.01). Among the 10 different formulation the formula F2 shows (6.85±0.01) compatible pH value with skin. The spreading diameter(c.m) of the prepared hydrogel was found to be in the range of (3.9±0.02) to (5.52±0.01). Among the 10 formulation the formula F2 shows the highest spredability ((5.52±0.01). Viscosity of the prepared formulation was measured using brook field viscometer viscometer with spindle number 7 at 10rpm at room temperature. Formula shows viscosity in the range (16933±2.5) to (15437±1.5). From these data concluded that formula F1 and F2 shows excellent extrudability and other shows good extrudability, and all formulated hydrogel show good washability.

Table No 10: Swelling index of Hydrogel formulation.

Sl.No	Time (hr)	Swelling index(%)				
		F1	F2	F3	F4	F5
1	1	14.18 ± 0.06	16.27 ±0.04	11.25 ±0.03	13.45 ±0.03	12.66 ±0.02
2	2	19.26±0.01	22.61 ±0.04	18.24 ±0.01	19.56 ±0.02	16.87 ±0.02
3	3	26.25 ± 0.02	31.34 ±0.01	23.47 ±0.02	25.66 ±0.02	23.56 ±0.02
4	4	33.66 ± 0.02	39.25 ± 0.02	29.62 ±0.02	32.46 ±0.02	28.21 ±0.02
5	5	38.24 ± 0.01	46.47 ±0.02	36.66 ±0.03	39.14 ±0.02	31.57 ±0.01
6	6	43.57 ± 0.02	52.37 ±0.01	39.14 ±0.03	42.47 ±0.02	37.37 ±0.01
7	7	49.6±0.02	59.24 ±0.01	44.27 ±0.01	47.55 ±0.02	41.38 ±0.02
8	8	54.15±0.03	62.26 ±0.02	46.65 ±0.02	52.21 ±0.02	45.70 ±0.01
9	9	58.74 ± 0.01	66.95 ±0.01	52.45 ±0.03	56.36 ±0.02	49.64 ±0.02
10	10	63.13±0.01	70.17 ±0.02	55.70 ±0.01	61.26 ±0.02	52.46 ±0.02

Sl.No	Time (hr)	Swelling index(%)				
		F6	F7	F8	F9	F10
1	1	15.36 ±0.02	10.47 ±0.02	12.56 ±0.02	11.35 ± 0.02	9.25 ±0.02
2	2	18.13 ±0.02	13.56 ±0.02	19.75 ±0.02	17.19 ±0.02	12.5 ±0.01
3	3	20.13 ±0.01	18.16 ±0.02	25.64 ±0.02	23.66 ±0.01	16.85 ±0.01
4	4	24.94 ±0.01	23.64 ±0.03	31.34 ±0.01	27.47 ±0.02	15.05 ±0.01
5	5	28.75 ±0.02	27.46 ±0.03	36.56 ±0.02	35.17 ±0.01	26.95 ±0.01
6	6	31.27 ±0.02	32.76 ±0.02	42.16 ±0.03	39.47 ±0.02	31.84 ±0.02
7	7	37.45 ±0.03	39.75 ± 0.02	46.67 ±0.02	41.26 ±0.02	35.65 ±0.02
8	8	42.25 ±0.03	43.65 ±0.01	51.17 ±0.02	46.85 ±0.02	39.44 ±0.02
9	9	44.76 ±0.01	48.65 ±0.02	56.34 ±0.02	48.53 ±0.01	42.64 ±0.02
10	10	47.51 ±0.02	50.25 ±0.03	61.86 ±0.02	52.55 ±0.01	44.33 ±0.02

The swelling index of the prepared hydrogel was found to be in the range of (70.17±0.02) to (44.33±0.01).Among the 10 formulation the formula F2 shows the highest swelling index (70.17±0.02).

Table No 11: Antioxident study of Hydrogel formulation.

Sl No	Concentration (µg)	IC_{50} (µg/ml)										
		Std	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1	20	18.22 ±0.01	24.23 ±0.02	22.06 ±0.02	26.74 ±0.01	25.57 ±0.02	24.86 ±0.01	25.16 ±0.02	26.23 ±0.01	23.14 ±0.01	24.61 ±0.02	26.35 ±0.02
2	40	26.31 ±0.01	31.15 ±0.02	30.74 ±0.02	35.26 ±0.01	34.27 ±0.01	32.74 ±0.01	34.26 ±0.01	33.28 ±0.02	31.28 ±0.02	32.15 ±0.03	35.47 ±0.01
3	60	32.12 ±0.01	38.24 ±0.02	37.33 ±0.02	44.65 ±0.01	40.57 ±0.02	39.45 ±0.02	46.13 ±0.02	41.65 ±0.04	39.25 ±0.01	42.25 ±0.02	47.65 ±0.03
4	80	43.23 ±0.02	49.46 ±0.02	48.26 ±0.03	55.35 ±0.01	46.32 ±0.02	49.96 ±0.03	53.47 ±0.02	51.55 ±0.02	49.13 ±0.01	50.74 ±0.01	54.17 ±0.02
5	100	52.12 ±0.01	57.84 ±0.01	55.46 ±0.01	65.87 ±0.02	59.65 ±0.01	58.34 ±0.03	63.25 ±0.02	59.24 ±0.01	56.54 ±0.01	58.57 ±0.01	62.74 ±0.01

Invitro Antioxidant study was carried using Hydrogen peroxide scavenging assay antioxidant activity was expressed in terms of IC_{50} value. Antioxidant capacity IC_{50} and values are inversely proportional to antioxidant activity. Lowest IC_{50} value shows highest antioxidant

activity. As antioxidant capacity increases the wound healing also increases. IC_{50} Value of prepared hydrogel was measured at different concentration. Formula F2 shows lowest IC_{50} Value(55.46±0.01).

Based on pH, spredability, viscosity, swelling index, antioxidant study F2 was selected as the best formulation. Hence it was taken for diabetic wound healing activity.

Skin irritation study

Table No 12: Skin irritation study of hydrogel(F2).

Sl. No	Treatment	Day1	Day2	Day3	Day4	Day5	Day6	Day7
1	F2	A	A	A	A	A	A	A

The result shows that prepared formulation is free from dermatological reactions. It did not produce erythema and edema for 7 days when applied over skin.

Diabetic wound healing activity

Diabetic wound healing effect of hydrogel F2 was determined by excision wound model. The percentage wound closure was measured and tabulated.

Table no 13: Diabetic wound healing activity.

Treatment Group	Wound contraction (mm)			
	1st day	3rd day	7th day	11th day
Normal	0.83±0.05	0.73±0.1	0.36±0.1	0.23±0.05
Positive control	1.06±0.1	1±0.1	1.1±0.1	1.16±0.05
Standard (Megaheal ointment)	0.86±0.1	0.8±0.1	0.53±0.05	0.36±0.05
Hydrogel(F2)	0.9±0.05	0.8±0.1	0.66±0.05	0.53±0.05

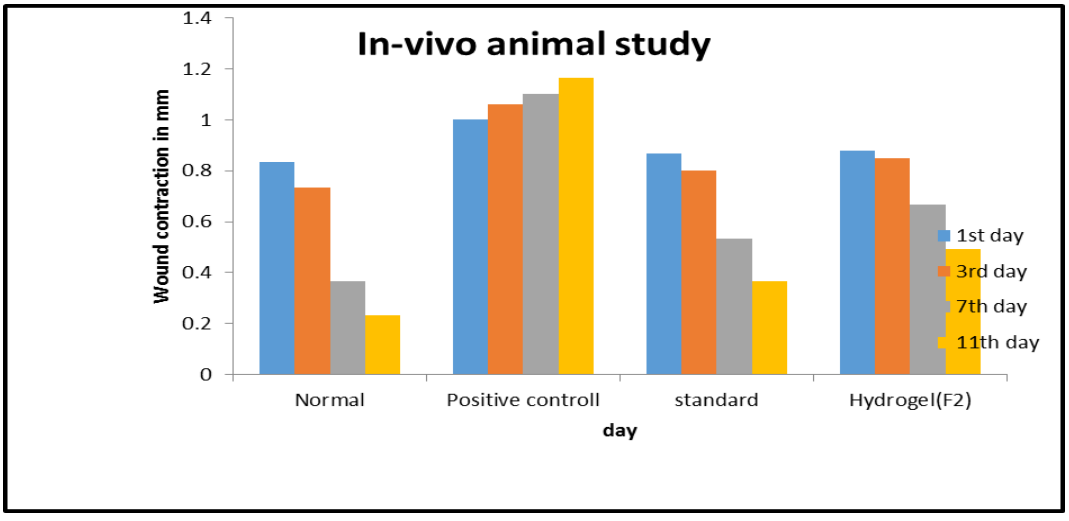
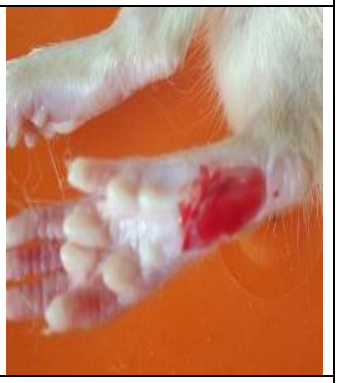
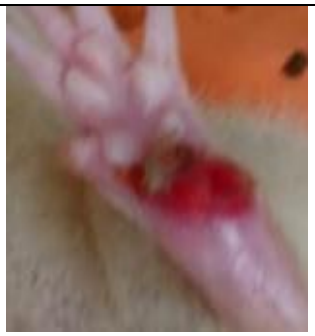
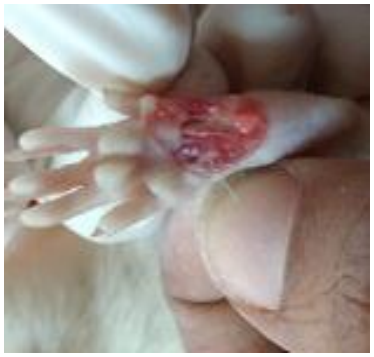
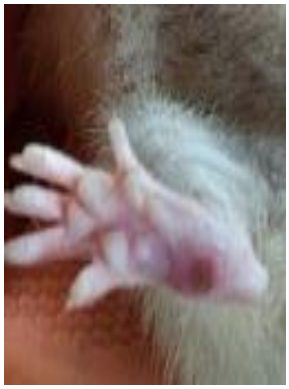
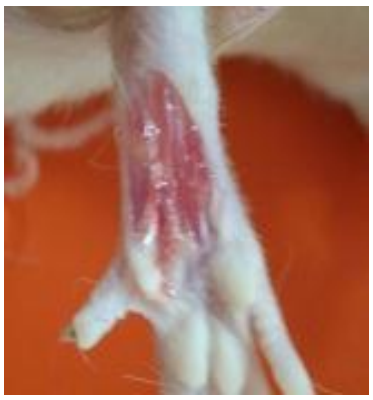


Figure no 8: Graphical representation of diabetic wound healing activity.

DAY	NORMAL	POSITIVE CONTROLL	STANDARD	TEST
ON 1st DAY				
ON 3rd DAY				
ON 7th DAY				
ON 11th DAY				

Diabetic wound healing study was done by using excision wound model. The diabetic was induced using streptozotocin. Rat were grouped into 4, normal, positive control, standard and test. The percentage wound closure was measured during 1st, 3rd, 7th, 11th day. On 11th day

percentage wound closure of normal, positive control, standard and test as (0.9±0.05),(0.8±0.1),(0.66±0.05),(0.43±0.05) respectively.

Hydrogel prepared from the ethanolic extracts of *Catharanthus roseus* and *Terminalia chebula* showed faster wound healing some what similar to standard(Megaheal ointment). Tannin and phenols present in the fruit of *Terminalia chebula* and alkaloid, tannin, triterpenoid present in the leaves of *Catharanthus roseus* fastness the epithelisation, tensile strength, and scavenge the free radicals responsible for delayed wound healing.

Stability study

Table No. 14: Stability studies of hydrogel (F2) 25-30°C±3°C.

Sl no	Evaluation parameter	After one month Observation	After two month Observation
1	**Colour**	Yellowish brown	Yellowish brown
2	**Appearance**	Clear and tanslucent	Clear and tanslucent
3	**Ph**	6.85±0.01	6.85±0.01
4	**Homogenity**	Homogeneous	Homogeneous
5	**Spreadability**	5.52±0.01	5.50±0.02
6	**Viscosity**	16933±2.5	16931±1.3
7	**Extrudability**	Good	Good

Table No. 15: Stability studies of hydrogel (F2) 2-8^{o}c±3°C.

Sl no	Evaluation parameter	After one month Observation	After two month Observation
1	**Colour**	Yellowish brown	Yellowish brown
2	**Appearance**	Clear and tanslucent	Clear and tanslucent
3	**Ph**	6.85±0.01	6.83±0.01
4	**Homogenity**	Homogeneous	Homogeneous
5	**Spreadability**	5.52±0.01	5.49±0.02
6	**Viscosity**	16933±2.5	16930±1.3
7	**Extrudability**	Good	Good

• Formulated gel F2 were kept at different temperature condition like room temperature(25°C±2°C**)** and refrigerator temperature (2-8^{o}c±3°C). It was confirmed that the developed hydrogel containing plant extracts was stable at the temperature range set for the studies. Based on pH, spredability, viscosity, swelling index and antimicrobial study F2 was selected as the best formulation. Which was also supported by the stability study.

CONCLUSION

Hydrogels are viscous semi-solid preparation, formed by the combination of one or more hydrophilic polymers. They make the applied site moister. It increases the amount of oxygen penetration and helps in wound healing. The sagas in ancient India, developed these magic remedies, without the aid of technological advances. Herbal medicine have better patient compliance because of their long term curing capacity and absence of side effects. Now a days demand of herbal preparation increases in world market The present work was aimed to a comparative study on different polymers for the preparation of hydrogel containing ethanolic extracts of *Catharanthus roseus, Terminalia chebula* and fresh aloe vera gel. On the basis of all parameter(pH, spredability, viscosity, swelling index, antioxidant study, Invivo animal study) F2 was selected as the best and optimised formulation. Hence concluded that carbopol based hydrogel containing ethanolic extracts of *Catharanthus roseus, Terminalia chebula* and fresh aloe vera gel found to be very effective for the treatment of diabetic wound healing.

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REFERENCES

1. AN Singab, O eldahshan. Medicinal Importance of Herbs & Spices. *Med Aromat Plants*, 2015; 4(4): 1-2.
2. Manta saxeena, Jyothi saxeena Rajeev Nema, Dharmendra Singh and Abhishek Gupta. Phytochemistry of medicinal plant. *Journal of pharmacognosy and phytochemistry*, 2013; 1(6): 168-182
3. Wade D. Aumiller and Harry Anderson Dollahite. Pathogenesis and management of diabetic foot ulcers. *Journal of the American Academy of Physician Assistants*, 2015; 28(1): 28-34.
4. Kleopatra Alexiadou and John Doupis. Management of Diabetic Foot Ulcers. *Diabetes Therapy*, 2012; 3(1): 4.
5. P Sharad.Pendsey. Understanding diabetic foot. International Journal of Diabetes in Developing Countries, 2010; 30(2): 75–79.

6. Leila Yazdanpanah, Morteza Nasiri and Sara Adarvishi. Literature review on the management of diabetic foot ulcer.*World J Diabetes*, 2015; 6(1): 37-53.
7. SuddhasuchiDas, B.AmitSharangi Madagascar periwinkle (*Catharanthusroseus*L.): Diverse medicinal and therapeutic benefits to Humankind.*Journal of Pharmacognosy and Phytochemistry*, 2017; 6(5): 1695-1701.
8. S.Gajalakshmi, S.Vijayalakshmiand V. Devirajeswari. Pharmacological activities of *catharanthusroseus*: a perspective review. *Int J Pharm Bio Sci*, 2013; 4(2): 431–439.
9. Monika Sain, VandanaSharma.*Catharanthusroseus*(An anti-cancerousdrug yielding plant) - A Review of Potential TherapeuticProperties.2013.*International Journal of Pure & Applied Bioscience*, 2013; 1(6): 139-142.
10. Anurag Singh, Pramod Kumar Singh and Rakesh Kumar Singh. Antidiabetic and Wound Healing Activity of *Catharanthusroseus*L. In Streptozotocin-Induced Diabetic Mice. *American Journal of Phytomedicine and Clinical Therapeutics*, 2014; 2(6): 686-692.
11. R.Rathinamoorthy, G.Thilagavathi.TerminaliaChebula - Review on Pharmacological and Biochemical Studies. *International Journal of PharmTech Research*, 2014; 6(1): 97-116.
12. PC Dodke, Pansare TA.2017.Ayurvedic and Modern aspect of *Terminaliachebula* Retz. *Haritaki* An Overview.2017. *International Journal of Ayurvedic& Herbal Medicine*, 2017; 7(2): 2508-2517.
13. N.TensinghBaliah, A.Astalakshmi.2014. Phytochemical analysis and antibacterial activity of extracts from *Terminaliachebula* Retz. *Int. J. Curr. Microbiol. App. Sci*, 2014; 3(3): 992-999.
14. N.N Azwanida.A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation.*Asian pacific journal of tropical medicine*, 2015; 4(3): 2-6.
15. R. Randall Wickett and Marty O. Visscher. Structure and function of the epidermal barrier. *American journal of infection and control*, 2006; 34(10): 98-110.
16. André Luís Morais Ruela, Aline Gravinez Perissinato, Mônica Esselin de Sousa Lino, Paula Silva Mudrik and Gislaine Ribeiro Pereira. Evaluation of skin absorption of drugs from topical and transdermal formulations. Braz. J. Pharm. Sci, 2016; 52(3).
17. ShivanandaNayak. Influence of Ethanol Extract of *Vincarosea*on Wound Healing in Diabetic Rats.*Online Journal of Biological Sciences*, 2006; 6(2): 51-55.
18. V.D.Jadhav, G.Talele Swati, A.Bakliwal Akshada and G.N Chaudhari. Formulation And Evaluation Of Herbal Gel Containing Leaf Extracts Of Tridax Procumbens. Journal of Pharmaceutical and Bio Sciences, 2015; 3(1): 65-72.

19. T.Regupathiand,K.Chitra.Physicochemical Analysis of Medicinal Herbs, *Eclipta Alba* (L.) Hassk and *Lippia Nodiflora* (Linn.). *International Journal of Pharmaceutical and Phytopharmacological Research Int. J. Pharm. Phytopharmacol. Res*, 2015; 4(4): 249-251.
20. Sumitra Singh and Bhagwati Devi Rohilla.Formulation and evaluation of herbal gel from different parts of cyamposistetragonoloba (l.) Taub. For wound healing, 5(3): 740-752.
21. Fedalicashishtoppo,Rajesh sing pawar, development optimisation and evaluation of different herbal formulations for wound healing.*International journal of pharmacy and pharmaceutical sciences*, 2015; 7(3): 447-450.
22. P.V Radhika and K.V Arun Kumar.Hemigraphiscolorata (blume) and glycyrrhizaglabra (linn) hydrogel for wound healing and antiinflammatory activity.*World journal of pharmacy and pharmaceutical sciences*, 2017; 6(2): 902-923.
23. R. Bhramaramba, sudheerbabu, Ch. Divya and Naga Deepthi. Formualtion and evaluation of herbal gel containing *Terminaliachebula* leaf extracts. *Scholar academic journal of pharmacy*, 2015; 4(3): 172-176.
24. Sudipta Das, Pallab K. Haldar, GoutamPramanik,Formulation and Evaluation of Herbal Gel Containing Clerodendroninfortunatum Leaves Extract. *Int.J. PharmTech Res*, 2011; 3(1): 140-143.
25. Dr. HemamaliniBalaji, Versatile Therapeutic effects of Vincarosea Linn.*International Journal of Pharmaceutical Science and Health Care*, 2014; 4(1): 59-75.
26. S. Gajalakshmi, vijayalakshmiand devirajeswari, pharmacological activities of catharanthusroseus: a perspective review. *International Journal of Pharma and Bio Sciences*, 2013; 4(2): 431–439.