Research Article

Preparation and evaluation of polyherbal hydrogels formulation for diabetic foot ulcer

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ABSTRACT

Aim: The aim of the present study was to prepare and evaluate the polyherbal hydrogel formulation for diabetic foot ulcer and related infections. **Materials and Methods:** Polyvinyl alcohol is used due to its proven biocompatibility, non-irritancy, and non-immunogenicity. Borax is used as a chemical cross-linker in concentrations ranging from 4 to 20% w/v. Chemical cross-linking is used for the preparation of hydrogels. The characterization of herbal extracts (melting point, infrared spectroscopy, and ultraviolet-visible spectroscopy) was also done. **Results:** Increasing concentrations of borax decreased the swelling ratio but increased gel fraction value which is indicative of increase gel strength. *In vitro* release study, among the three extracts, the neem released was found to be more than *Aloe vera* and Guduchi. **Conclusion:** All the studies done clearly indicate that the optimized formulation has all the properties requisite of an ideal wound dressing with added benefit of excellent wound healing. This formulation holds promise in the future of the treatment of diabetic foot ulcers which may or may not be infected.

Keywords: *Azadirachta indica*, cross-linking, diabetic foot ulcer, hydrogel

Introduction

Hydrogels have been defined as two or multicomponent systems consisting of a three-dimensional network of polymer chains and water that fills the space between macromolecules. Depending on the properties of the polymer (polymers) used, as well as on the nature and density of the network joints, such structures in an equilibrium can contain various amounts of water; typically in the swollen state, the mass fraction of water in a hydrogel is much higher than the mass fraction of polymer. In practice, to achieve high degrees of swelling, it is common to use synthetic polymers that are water soluble when in non-cross-linked form.[1] Hydrogels may be synthesized in a number of "classical" chemical ways. These include one-step procedures such as polymerization and parallel cross-linking of multifunctional monomers, as well as multiple step procedures involving the synthesis of polymer molecules having reactive groups and their subsequent cross-linking, possibly also by reacting polymers with suitable cross-linking agents. The polymer engineer can design and synthesize polymer networks with molecular-scale control over structure such as cross-linking density and with tailored properties such as biodegradation, mechanical strength, and chemical and biological response to stimuli.[2]

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Healing benefits

Due to the moisture provided to the wound from the hydrogel dressing, common healing phases such as granulation, epidermis repair, and the removal of excess dead tissue become simplified. In addition to aid the wound treatment stages, the cool sensation provided by the hydrogel to the wound offers relief from pain for at least 6 h. When hydration is provided for the wound bed, discomfort experienced from changing the dressing becomes reduced, and the risk of infection also becomes decreased.[3] Hydrogels were first used as wound dressing by Rosiak, and nowadays, they are considered as

one of the best topical formulations for wound healing in spite of the availability of the number of other dressings such as hydrocolloids, alginate dressings, and foams. This can be attributed to the following reasons:

- Promoting moist healing and autolytic debridement (most important factor)
- Control of drug dosage release
- Easy replacement
- Transparency to allow healing follow-up
- Barrier against bacterial infection
- Excellent oxygen permeability
- Cool the wound surface immediately reducing pain.

These mentioned factors make the dressing patient compliant.[4]

Materials and Methods

Materials and equipment

Authentication of plants

Plants such as neem, "*Aloe vera,*" and Guduchi were collected from Surajpur, Teh. Baddi, and Dist. Solan (Himachal Pradesh [H.P]) and were authenticated at the National Institute of Science Communication and Information Resources, New Delhi. Due to lack of facilities, instead of using the plants, commercially available extracts of these plants were obtained. These extracts were obtained as gift samples from Sirmour Herbolife. These extracts were evaluated and used for formulation development purpose. Polyvinyl alcohol (PVA) from CDH Laboratory reagent, borax from standard deviation Fine Chem Limited, and polyethylene alcohol from Molychem Limited, Mumbai (India) were purchased. All other laboratory chemicals used in the study were of analytical reagents grade and several equipments employed in the formulation of herbal patches were magnetic stirrer, Petri dish, ultrasonic cleaner, electronic balance, pH meter, ultraviolet (UV)-visible spectrophotometer, Fourier transformed infrared (FTIR), tray drier, and hot air oven.

Characterization of herbal extracts

Herbal extracts identification

Herbal extracts were obtained from Sirmour Herbolife Pvt. Ltd., H.P, India. Obtained extracts were identified according to standard procedures.[5]

Physical appearance

Physical appearance of procured extracts was noted by visual observation.

Melting point determination

Melting point was determined using digital capillary apparatus. A small amount of drug was filled into one-sided sealed capillary and was placed in the melting point apparatus along with calibrated thermometer. This test was performed in triplicate to observe the melting point range.[6]

Active component-excipient interaction study

IR study was performed for identification and structural analysis of the herbal extracts using FTIR spectrophotometry. The KBr disc technique was employed using 1 mg of herbal extracts powder in 100 mg of spectroscopic grade dried KBr. Mixture was grounded into a fine powder using an agate mortar/pestle and compressed into KBr disc under a hydraulic press at 10,000 psi. Each KBr disc was scanned 32 times at 4 mm/s at a resolution of 2 cm^{-1} over a wavenumber region of 400-4000 cm^{-1} and characteristic bands were recorded. Further results were compared with standard peaks available in literature.[7,8]

UV-visible spectrophotometric method

Estimation of herbal extracts in pharmaceutical formulation was done by plotting its calibration curve in phosphate-buffered saline (PBS) (pH 7.4).

Sample preparation

1. Preparation of PBS (pH 7.4)
   It was prepared by dissolving 2.7 gm of potassium dihydrogen phosphate and 0.8 g of sodium chloride in 100 ml of distilled water.
2. Preparation of standard stock solution
   One mg/ml stock solutions of herbal extract were prepared in PBS (pH 7.4) which was further diluted to 100 µg/ml.
3. Preparation of working solutions
   Working solution was prepared in the range of 1–6 µg/ml in PBS (pH 7.4). Absorbance of these solutions was recorded at λ max of 220 nm, 211 nm, and 265 nm for (write down the name of extract here for which you have used these wavelengths such as for "*A. vera*," neem, and Guduchi, respectively) against blank using UV-visible spectrophotometer.

Table 1: Preparation of hydrogel with different concentrations of PVA and borax

Formulation No.	Quantity of 4% w/v PVA (ml)	% of 10 ml of borax solution (%)	PVA: borax
F1	100	4	1:0.1
F2	100	8	1:0.2
F3	100	12	1:0.3
F4	100	16	1:0.4
F5	100	20	1:0.5
F6	50	12	1:0.3
F7	50	20	1:0.5

PVA: Polyvinyl alcohol

Table 2: Melting point of herbal extracts

Extract	Reference	Experimental
Aloe vera	148°C	146°C
Neem	160°C	157°C
Guduchi	178°C	175°C

Table 3: Swelling ratio results of the selected formulations

Formulation No.	Initial weight (W_a) (g)	Final weight (W_s) (g)	Swelling ratio (W_s/W_a) (%)
F3	70.16	70.29	530
F4	58.51	58.68	466
F5	58.51	58.60	400

Table 4: Gel fraction of the selected formulations

Formulation No.	Initial weight (W_o) (mg)	Final weight (W_e) (mg)	Gel fraction (W_e/W_o) (%)
F3	1354.2	514.4	37.9
F4	1509.3	739.1	48.9
F5	1182.7	720.3	60.9

Formulation of hydrogel

Method of preparation of hydrogel

PVA/borax/polyethylene glycol (PEG) 400 hydrogels were prepared by chemical cross-linking. Briefly, 4% PVA solution was prepared by dissolving 4 gm in 100 ml of distilled water while 4, 8, 12, 16, and 20% concentration of borax were prepared by dissolving 4, 8, 12, 16, and 20 g of borax in 100 ml of water. They were then mixed in the weight ratio of 10:1 (19), 10:2, 10:3, 10:4, and 10:5, respectively (all were without drug). For increase in flexibility, plasticizers, namely, glycerine and PEG 300 were added in the percentage of 10–50% w/w of the final optimized formulation before the addition of cross-linker. Herbal extracts of neem (1%), "*A. vera*" (0.5%), and Guduchi (2%) were added directly into the PVA solution [Table 1].

Procedure for preparing a hydrogel loaded with herbal extracts

1. About 4, 10, and 16% w/v concentration of PVA were prepared by dissolving 4, 10, and 16 g of PVA in 100 ml of water
2. About 4, 8, 12, 16, and 20% w/v concentration of borax were prepared by dissolving 4, 8, 12, 16, and 20 g of borax in 100 ml of water
3. For 4% PVA solution, 10 ml of 4% borax solution was added with continuous stirring resulting in immediate formation of hydrogel of ratio 1:01
4. Same procedure was repeated for remaining concentration of borax as well-forming hydrogels of ratio 1:02 (8% borax), 1:03 (12% borax), 1:04 (16% borax), and 1:05 (20% borax)
5. For 10 and 16% w/v PVA solution and 2.5% w/v solution of borax were used and prepared in the same way as mentioned above
6. Additional ingredients, namely, glycerine and PEG 400 were used in concentration ranging from 10 to 50% (w/w) which were added to PVA solution before the addition of borax solution
7. Herbal extracts neem (1% w/w), *A. vera* (0.5% w/w), and Guduchi (2% w/w) were added the selected formulations and in the final optimized formulations.[9]

Optimization of hydrogels

The various optimization parameters include gel fraction determination, swelling ratio determination, water vapor transmission (WVT) test, and *in vitro* drug release studies.

Swelling ratio

Pieces of hydrogel (2 cm × 2 cm) were dried for 6 h at 60°C under vacuum. Further, they were dipped in 10 ml of PBS (pH 7.4) and kept in Petri dishes for 24 h until a constant weight was obtained. After the removal of any soluble part, they were then again dried at 60°C for 6 h. It was calculated as follows:

$$\text{Swelling ratio} = \frac{W_s}{W_a} \times 100$$

Where,

W_s = Weight of hydrogel sample after drying for 6 h at 60°C
W_a = Weight of hydrogel sample after soaking in PBS at 37°C.

Gel fraction

Pieces of hydrogel (2 cm × 2 cm) were dried for 12 h at 60°C under vacuum. They were then dipped in 10 ml of PBS (pH 7.4) and kept in Petri dishes at 37°C. It was calculated as follows:

$$\text{Gel fraction} = \frac{W_e}{W_o} \times 100$$

Where,
W_e = Weight of hydrogel sample after soaking and drying at 60°C
W_o = Weight of hydrogel sample after drying at 60°C.

WVT test

This test was performed by partial drying of a piece of hydrogel sample and placing it over a dish of internal diameter of 7 cm containing 10 ml water (100% RH). The whole set up was placed in a desiccator containing calcium chloride (0% RH). It was weighed at the 1st and 2nd h and was calculated as follows:

$$\text{WVT} = \frac{(W2 - W1)}{S} \times 24$$

Where, W1 and W2 are the weights of the whole cup at the 1st and 2nd h.

In vitro drug release

For the study of drug release profile, various membranes are used which can be natural or synthetic. Natural membranes such as inner layer of

egg, peach, tomato, and onion are used for drug permeation studies. Egg membrane is collected by placing an egg in concentrated 3 M solution of HCl. Once the foam disappears and bubbling stops leftover substance is eggshell with yolk. Remove the eggshell membrane from the HCl solution and washed the membrane with PBS (pH 7.4). This membrane is used for the *in vitro* studies. Exact amount (1 g) of formulation was placed on egg membrane. Egg membrane was sealed and placed into 50 ml of PBS in a beaker. The whole set up was placed on a magnetic stirrer at 37°C at 100 rpm. At predetermined time intervals, 5 ml of the sample was taken out and replaced with fresh PBS. The drug concentration was determined by UV-visible spectroscopy (Shimadzu UV-1700) at 200–400 nm.[10]

Microbial contamination test

As the herbal hydrogels are more prone to microbial attack, the formulation was subjected for microbial contamination test. Sterilized nutrient agar was prepared and then a small quantity of the formulated 2% herbal hydrogel was spread on it and incubated for 24 h at 37°C. The growth for any microorganisms was observed.[11]

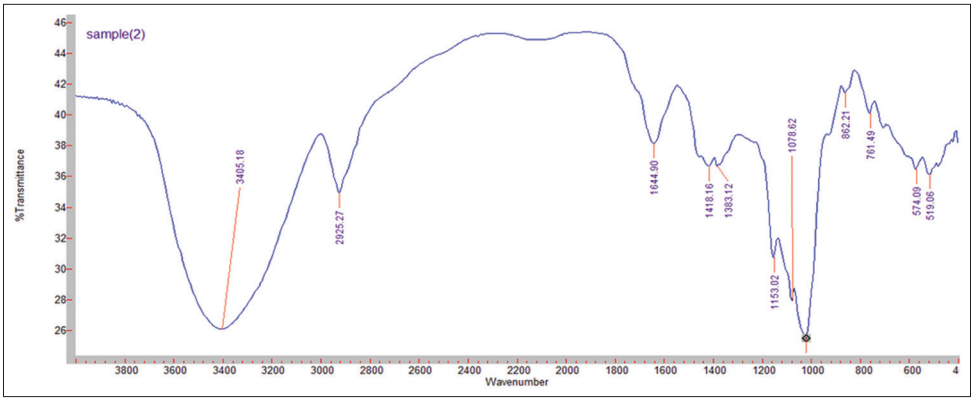

Figure 1: Infrared spectra of *Aloe vera* extract

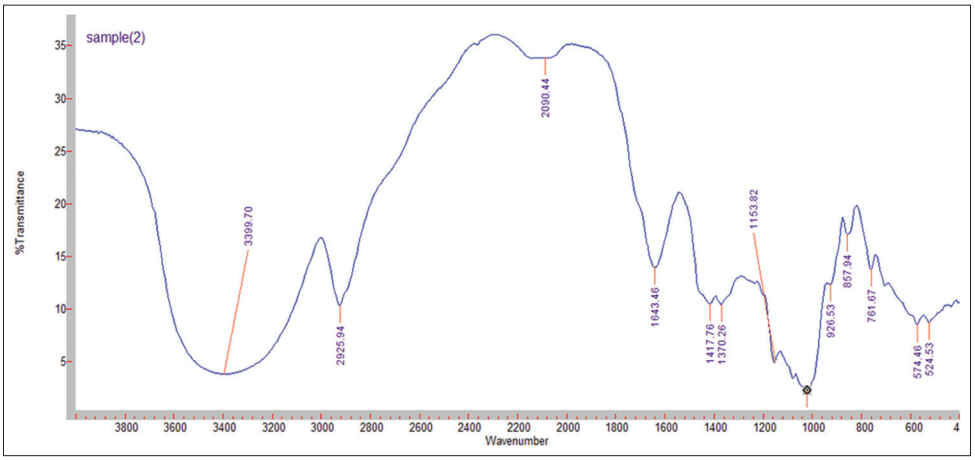

Figure 2: Infrared spectra of neem extract

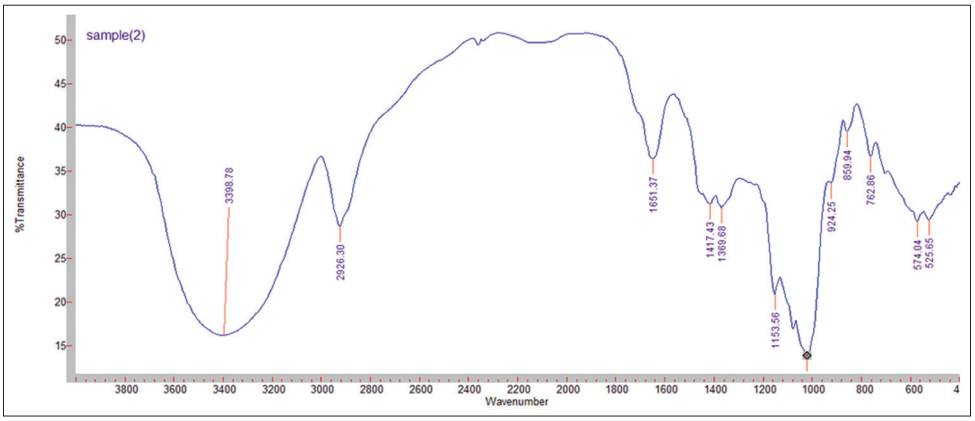

Figure 3: Infrared spectra of Guduchi extract

Skin irritation test

Mark an area (1 sq.cm) on the left-hand dorsal surface of goat. The formulation was applied to the specified area and time was noted. Irritation was checked if any for regular intervals up to 24 h and reported.

Results and Discussion

Herbal extracts identification

Herbal extracts were identified on the basis of its physical appearance, melting point, and IR spectra. Based on the results of characterization, the study of the herbal extracts was confirmed.

Physical appearance

Herbal extracts of *A. vera*, neem, and Guduchi were visually observed and were found to be pale brown-colored powder.

Melting point

The melting point was found to be 148, 160, and 178°C for *A. vera*, neem, and Guduchi extract, respectively, which are as per the reported melting point. Table 2 gives the details of melting point observed.

IR analysis

IR spectrum of *A. vera*, neem, and Guduchi is shown in Figures 1-3, respectively. Observed peaks in IR spectrum were found to be in concordant with functional groups present in structure of *A. vera*, neem, and Guduchi. Figures 1-3 show the frequency of observed bands and its interpretation. Purity of procured sample was confirmed from its IR spectrum. These results confirmed the identity and purity of both procured extracts.

Standard curve of herbal extracts

To obtain the calibration curve, different concentrations of *A. vera*, neem, and Guduchi were dissolved in PBS (pH 7.4) and absorbance was measured. Figures 4-6 explain the calibration curve of *A. vera*, neem, and Guduchi in PBS (pH 7.4) with correlation coefficient $r^2 = 0.998$ (*A. vera*), $r^2 = 0.991$ (neem), and $r^2 = 0.996$ (Guduchi). Results inferred that Beer's law was obeyed in concentration ranges of 2–10, 2–12, and 2–10 µg/ml *A. vera*, neem, and Guduchi, respectively.

Formulation development and optimization of herbal extracts loaded hydrogels

Hydrogel was prepared according to the procedure and was optimized on the basis of gel fraction, swelling ratio, and drug release studies. The pictures of the prepared formulations are shown in Figure 7.

Swelling ratio

Swelling ratio describes about the degree of cross-linking as well as gel strength. Very high swelling ratio or low swelling ratio is undesirable as high value indicates decreased gel strength and low cross-link density, whereas low swelling ratio indicates high cross-link density. Greater the cross-link density, lesser is the water-absorbing capacity.

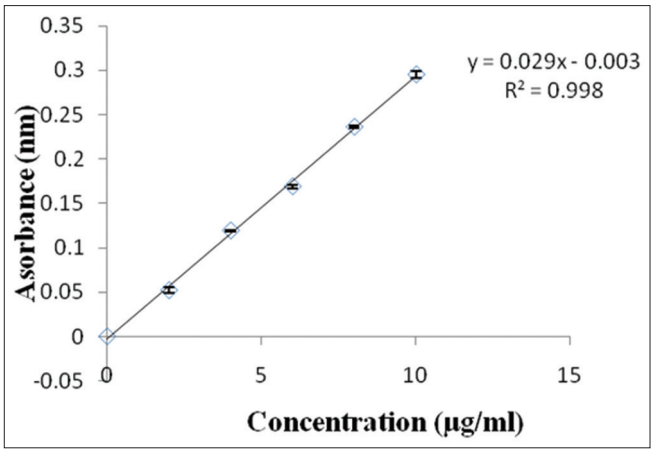

Figure 4: Calibration curve of *Aloe vera* in phosphate-buffered saline (pH 7.4)

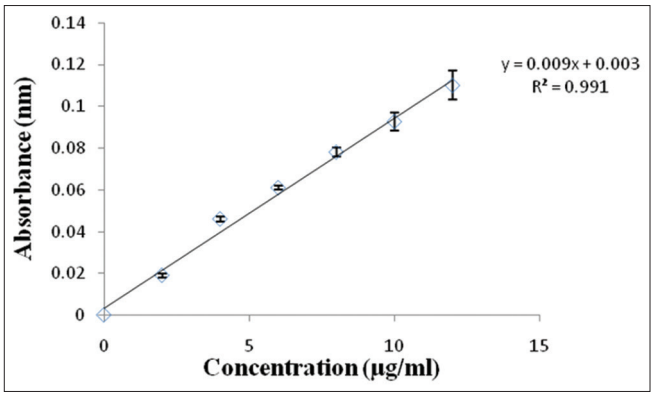

Figure 5: Calibration curve of neem in phosphate-buffered saline (pH 7.4)

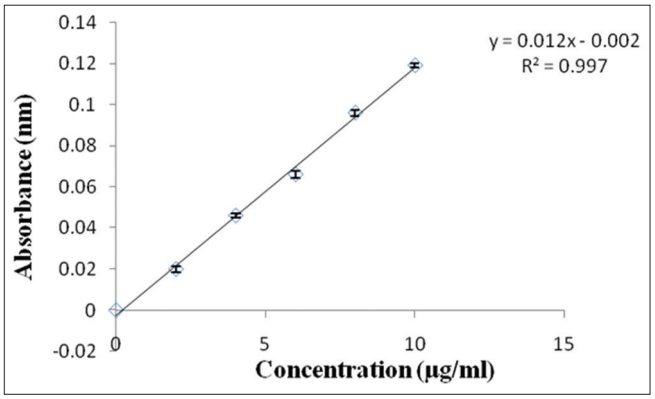

Figure 6: Calibration curve of Guduchi in phosphate-buffered saline (pH 7.4)

F3 had exceptionally high swelling ratio which indicated low cross-link density, while F4 and F5 depicted ideal swelling ratio [Table 3].

Gel fraction

Decrease in gel fraction corresponds to decreased gel strength but with increased flexibility. As already stated, F3 had low cross-link density and as per the result of the gel fraction had highest flexibility [Table 4]. Therefore, F4 and F5 were selected for further studies as they had desired flexibility (optimum gel fraction value) and cross-link density (swelling ratio value).

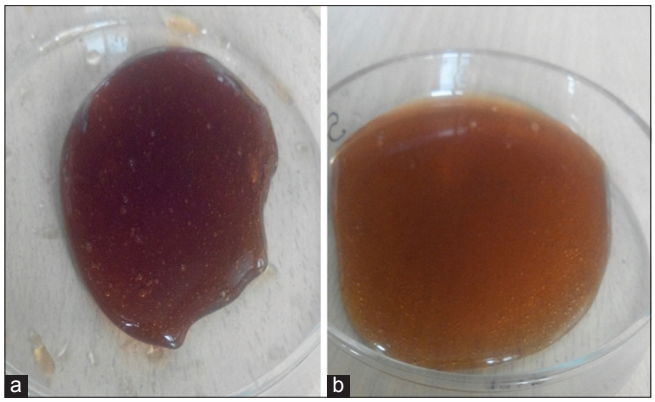

Figure 7: (a) Formulation F9 (b) formulation F10

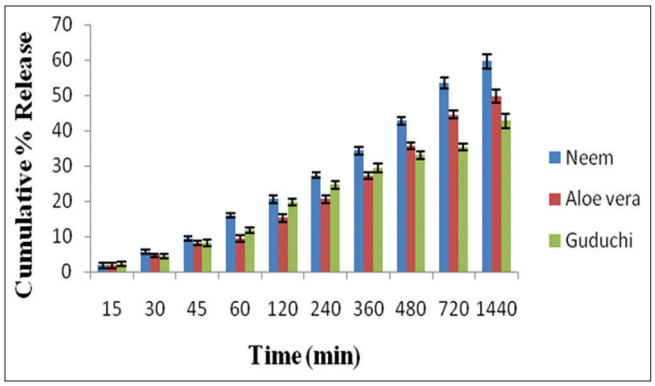

Figure 8: Cumulative percentage release of F8

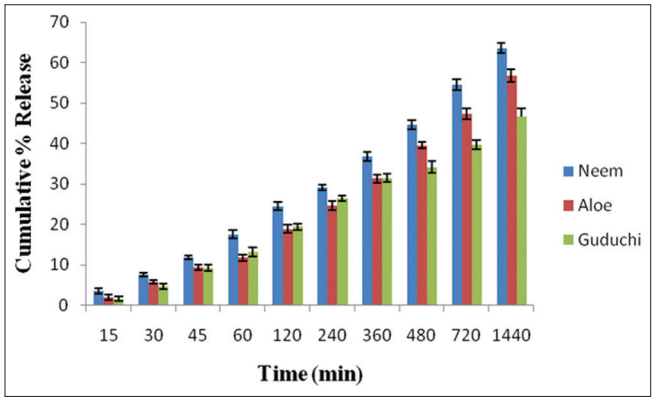

Figure 9: Cumulative percentage release of F9

In vitro drug release

Release studies were done in PBS (pH 7.4) for optimizing the formulation. F4 and F5 were loaded with 0.5%, 1%, and 2% (w/w) extracts (now termed F8 and F9) and drug release was checked. Figure 8 shows the percentage cumulative release of extract in 24 h of F8 (59.7% of neem, 49.8% *A. vera*, and 42.6% of Guduchi release).

Figure 9 shows the percentage cumulative release of extract in 24 h of F9 (63.33% of neem, 56.73% of *A. vera*, and 46.9% of Guduchi release); the mechanism for drug release in hydrogels is swelling followed by erosion/dissolution of formulation in the external environment. From the release curve of F8 and F9 clearly observed that F9 showed better drug release per hour as well as the percentage cumulative release in 24 h. This may be due to higher dissolution of the formulation due to which more amount of drug escapes into the outer environment.

WVT test

For an ideal wound dressing, the value of WVT rate (WVTR) lies between 2000 and 2500 g/m^2/day which is an indication of adequate level of moisture at the wound site needed to prevent dehydration and/or buildup of exudates. However, the WVT result of optimized formulation was not found in ideal range. For this test, F5 was partially dried in vacuum oven at 37°C. F5 had WVTR value of 4000 g/m^2/day, F8 had WVTR value of 4533 g/m^2/day, and F9 had WVTR value of 4800 g/ m^2/ day. Thus, F10 was finalized and extract was loaded with mentioned excipients and the extract loaded formulation was named as F12.

Conclusion

It can be concluded that the herbal drugs in the form of extracts can also be formulating the hydrogel for the treatment of diabetic foot ulcer. Hence, all the studies done so far clearly indicate that the optimized formulation has all the properties requisite of an ideal wound dressing with added benefit of excellent wound healing.

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