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Fabrication and evaluation of a medicated hydrogel film with embelin from *Embelia ribes* for wound healing activity

Hiral Shrimali¹, Uttam Kumar Mandal^{2†}, Manish Nivsarkar^{3†} and Neeta Shrivastava^{3*}

Abstract

Background: There has been huge interest among the researchers to incorporate a medicinally active compounds in hydrogel sheets for effective treatment of wound healing. This research work involves development and evaluation of medicated hydrogel sheet incorporated with embelin which has both antimicrobial and wound healing activity. Embelin was isolated from the fruits of *Embelia ribes* and characterized by various physical and analytical methods like melting point, UV/VIS spectroscopy, and HPTLC. The hydrogel sheets containing polyvinyl alcohol and polyethylene glycol was prepared by freeze-thaw technique, where isolated embelin was successfully incorporated within the sheet. The prepared hydrogel sheets were further characterized by *in vitro* drug release study, swelling capacities, gel fraction, water vapor transmission rate (WVTR), mechanical strength, and scanning electron microscopy (SEM) study. Finally, the optimized hydrogel with embelin was evaluated for its wound healing efficacy *in vivo* using excision wound model on Sprague–Dawley rats.

Results: The optimized hydrogel sheet had a composition of 5% PEG 400 and 10% PVA. It had acceptable in physico-chemical properties with respect to swelling capacities, gel fraction, water vapor transmission rate (WVTR), and mechanical strength. The release of the drug from hydrogel followed zero order kinetics with more than 80% drug release within 12 h. The *in vivo* studies on the Sprague–Dawley showed faster healing process with embelin loaded hydrogels as compared to the control and market formulation.

Conclusions: Sheet hydrogel with 0.2% embelin was found to have huge potential for moist wound healing activity.

Keywords: Wound healing, Hydrogel sheet, Polyvinyl alcohol, Polyethylene glycol, Embelin

Background

Apart from financial burden, wounds and particularly chronic wounds (that do not heal within 3 months) causes a lot of sufferings to patients in their social life. In the USA, chronic wound related complications affect 5.7 million patients, which costs an estimated 20 billion dollars annually [1]. Increased number of geriatric patients together with a huge volume of surgeries and increased awareness about wound care products among the presents have resulted such a steep growth of this

market. According to the Global Wound Care Market report, the Wound Care Market accounted a value of \$18.22 billion in 2016 and is projected to reach a value \$26.24 billion at the end of 2023. According to another recent past market survey with forecast for world market of wound management products until the year 2022, demand for wound management products is expected to grow at a compound annual growth rate (CAGR) of 6.7% (<https://www.psmarketresearch.com/press-release/wound-care-market>). As per that survey, China will form the fastest expanding wound management; however, the significant market growth will be experienced by North America (CAGR 7.1%), Asia pacific (CAGR 7.2%), Europe, and other regions. This has obviously drawn huge attention to the pharmaceutical manufacturers to develop patient-friendly wound care products.

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In general, a wound occurs when the integrity of any tissue is compromised (e.g., skin breaks, muscle tears, burns, or a bone fracture). A wound may be caused as a result of an injury from accident, a surgical procedure, an infectious disease or an underlying pathological condition [2]. So, it becomes very essential to manage such kind of wounds which may be achieved by the use of dressings. Traditionally, used dressings like cotton swabs and cotton gauzes suffer from the difficulties like they make the wound dry and also their removal is painful; thus, there has been always a genuine need for modern dressings which facilitate wound healing rather than just cover it. The modern dressings include hydrocolloids, films, alginate dressings, and hydrogels termed as occlusive dressings [3]. Apart from hydrogels, the other dressings have limitations like the inability to absorb excess wound exudates, adhesive may strip away newly formed epithelium, always require secondary dressing, and impermeable to oxygen. Thus, there is a need for a dressing which could eliminate these limitations or rather minimizes them.

Hydrogels are a class of polymer materials that can absorb large amounts of water without dissolving. They are cross-linked polymeric gels that have water-holding and non-adhering properties. They work on the principle of moist wound healing proposed by George Winter [4]. This has several advantages like decreased dehydration and cell death, increased angiogenesis, enhanced autolytic debridement, increased re-epithelialization, and decreased pain [5]. Few sheet hydrogels are available in international markets like Nu-gel[®], Vigilon[®], Flexigel[®], and Aquamatrix[®] [6], but they are all composed without any medically active agents in them. This has created huge interests among the researchers to incorporate a medicinally active compound in these hydrogel sheets. The present research work is based on the same approach where an attempt was made to prepare a sheet hydrogel containing a medicinally active compound embelin which was extracted from *Embelia ribes*, purified and characterized before the formulation. *Embelia ribes* is woody shrub belongs to the family Myrsinaceae, which is distributed in less dense moist deciduous forests of the Western Ghats, India, Sri Lanka, Malaysia, and South China [7]. It is commonly known as bidang, vidang, vavding, or vava vidang in the Indian Ayurvedic system. Embelin, the active constituent this plant, has anticonvulsant [8], antibacterial [9], antioxidant [10], and antifertility activities [11]. Apart from these activities, embelin has been reported for wound healing and antimicrobial activities [12, 13], and accordingly it was selected as active ingredient for this work.

Methods

Materials

Embelia ribes fruits were collected from the local market of Ahmedabad, India and authenticated by botanist at B.

V. Patel PERD Centre, Ahmedabad, India. The reference standard was obtained from RRL (Regional Research Laboratory) Jammu, India. Polyvinyl alcohol (MW 1, 95, 000) and polyethylene glycol 400 were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA) and Merck Limited (Mumbai, India), respectively. Petroleum ether, sodium bicarbonate, sodium di-hydrogen orthophosphate, potassium chloride, and sodium chloride were purchased from Qualigens Fine Chemicals, Mumbai, India. Calcium chloride was purchased from Merck Ltd, Mumbai, India. All the reagents were used as supplied by the suppliers without any further purification.

Isolation of embelin

The pure embelin was isolated from *E. ribes* by the method developed in our laboratory. Briefly, the fruits of *E. ribes* were powdered. The weighed amount of powdered drug (100 g) was taken in a conical flask. It was extracted with Petroleum ether for 4–5 times (4 × 100 ml). The extraction was carried out with intermittent heating at temperatures not exceeding 50 °C. The extract was filtered using a Whitman filter paper. After the complete extraction, the filtrates were pooled together and kept at 4 °C overnight. The obtained orange colored precipitate was washed with petroleum ether to remove the fatty impurities and further recrystallized with diethyl ether.

Characterization of embelin powder

The purity of the obtained embelin powder was confirmed by the following:

Melting point

For the determination of melting point, the isolated embelin was filled in the capillary tube which was sealed at one end. A similar procedure was followed for reference standard too. The capillary tubes containing the samples were placed in the Veego melting point apparatus and the instrument was switched on. The temperature at which the samples melted was noted.

UV-Vis spectroscopy

For the UV analysis, a sample of isolated embelin was prepared in methanol for which it was dissolved in methanol. The spectra of the resultant solution was taken using Shimadzu UV-2450. Similarly, a solution of the reference standard was also prepared in methanol and was subjected to spectral analysis.

Mass spectroscopy

The analysis of the mass of the isolated compound was performed by Mass Spectrometer (API 2000, AB Sciex) to confirm the identity of the compound. The reported molecular weight of embelin is 294 (Merck Index, page 3513).

High-performance thin layer chromatography

A sample solution of the reference standard and the isolated compound were subjected to HPTLC study. The samples were applied on the TLC plates using Linomat V auto sampler. The plate was developed in a mobile phase constituting of n-propanol: n-butanol: ammonia (4 N) in a ratio of 7:1:2 v/v in a twin trough TLC chamber which was pre-saturated with the mobile phase. The developed plate was dried and then was scanned in the CAMAG scanner using the software WINCATS. Spectra of reference standard and isolated embelin thus obtained were compared by spectral overlay method.

High-performance liquid chromatography

To further ensure the purity of the isolated embelin, and also for analysis of the released embelin from the formulation, a HPLC method was developed using a C₁₈ (Gemini Su, 250 × 460 mm, 5 μ) reverse phase column. The various ratios of methanol:water mobile phase were tried to resolve the peak shape and purity. Out of the various ratios, the mobile phase consisting of 80:20 (v/v) ratio of methanol:water (adjusted to pH – 9.02 using aq. ammonia) at a flow rate of 1 ml/min (PU 2080 plus pump) got good results and was selected for further experimentation. The samples for analysis were prepared in the mobile phase prior to the analysis. The column was allowed to pre saturate with the mobile phase; thereafter, the samples were injected using an autosampler (Jasco AS 950). The peak was detected using a photodiode array detector (Jasco MD 2010 plus multi wavelength detector).

Preparation of hydrogel sheets

The hydrogel sheets were prepared by freeze-thaw (F-T) technique [14] according to the compositions mentioned in Table 1. PVA was used as a gel forming polymer. PVA and PEG 400 were dissolved in alkaline distilled water (pH 7.7). The solution was heated on a water bath for 1 h with continuous stirring to dissolve the PVA completely. Then embelin powder (0.2%) was added in it and the final solution was poured into a Petri dish plate of 8 cm in diameter. The plate was frozen at – 80 °C for 16 h and then thawed at room temperature for 8 h for three consecutive cycles which resulted in the final hydrogel sheet [15, 16].

Table 1 Various compositions of PVA and PEG 400 used to prepare the hydrogel sheets

Ingredients	I	II	III	IV	V	VI	VII	VIII	IX
PVA	8	10	12	8	10	12	8	10	12
PEG 400	2.5	2.5	2.5	5	5	5	7.5	7.5	7.5
Water qs	100	100	100	100	100	100	100	100	100

*Each ingredient was added w/w %

Characterization of hydrogel sheets

Gel fraction

After three freeze-thaw (F-T) cycles, the hydrogel sheets were dried for 6 h at 50 °C in an oven (W_0). They were then soaked in distilled water for 24 h until a constant weight was reached. The hydrogels were then dried again at 50 °C in oven (W_e). The gel fraction percentage was calculated by the following equation [17].

$$\text{Gel fraction} = \left(\frac{W_e}{W_0} \right) \times 100 \quad (1)$$

where W_0 and W_e are the weights of hydrogel samples dried for 6 h at 50 °C before and after soaking, respectively.

Swelling index

The pieces of hydrogel sheets (1 cm × 1 cm) were dried at 60 °C in oven for 12 h (W_a). They were then soaked in a simulated wound fluid (SWF) pH 7.7 at 37 °C (W_s). The swelling index percentage was calculated using the following equation [17, 18].

$$\text{Swelling index} = \left(\frac{W_s}{W_a} \right) \times 100 \quad (2)$$

where W_a and W_s were the weights of hydrogel samples dried for 12 h at 60 °C and soaked in PBS at 37 °C, respectively.

Water vapor transmission rate

The water vapor transmission tests were performed using the JIS 1099A standard method [16]. A round piece of hydrogel was mounted on the mouth of a cup (1.8 cm in diameter) containing 12.8 g of CaCl₂ and placed in an incubator of 75% RH at 40 °C.

The water vapor transmission rate (WVTR) was determined as follows:

$$\text{WVTR (g/m}^2 \times \text{day)} = \left(\frac{W_2 - W_1}{S} \right) \times 100 \quad (3)$$

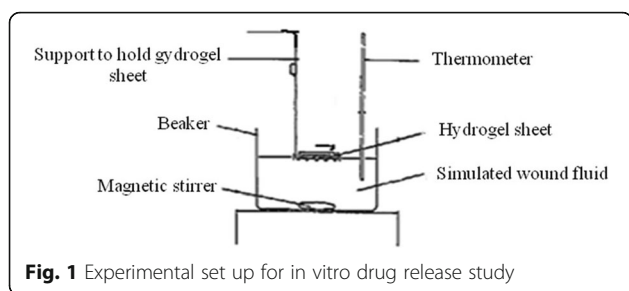
where W_1 and W_2 were the weights of the whole cup at the first and second hours, respectively, and S was the transmitting area of the sample.

Surface morphology

The surface morphology of the samples was investigated by scanning electron microscopy (SEM JSM–5610 LV). After 24 h of dissolution study, the hydrogel sheets were dehydrated by drying them in oven and were then subjected to SEM study [15].

Mechanical properties

The tensile strength and breaking elongation of hydrogels were determined using a tensile test machine (QTS



Brookefield Texture Analyzer). After three F-T cycles, the hydrogel sheets were cut into a rectangular shape (6-cm long and 1.5-cm wide), and they were subjected to the mechanical analysis at a stretching rate of 60 mm/min with an initial load of 5 g [18].

In vitro drug release study

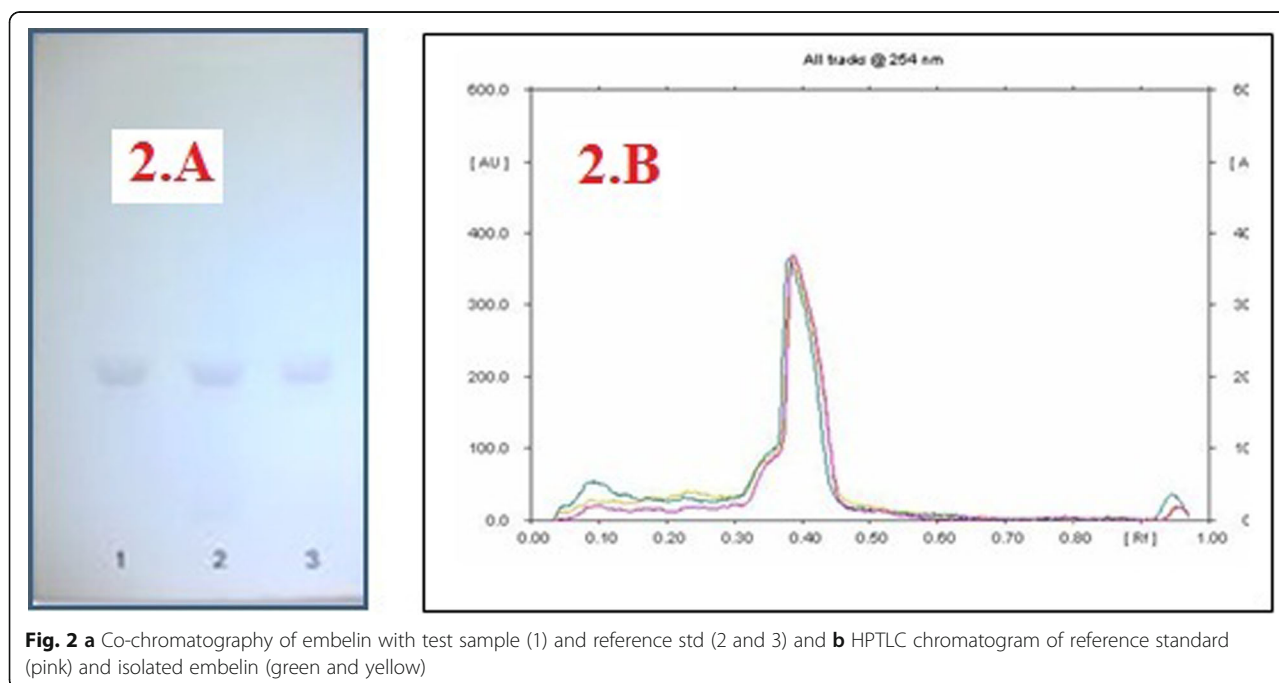
In vitro drug release of the F-T hydrogel sheets was carried out using a modified dissolution test instrument (Fig. 1) designed in-house. The method followed was total immersion method [19]. Hydrogel sheets were immersed with the help of a support in a beaker containing 20 ml of simulated wound fluid (142 mM sodium chloride and 2.5 mM calcium chloride in water). The beaker was placed on a magnetic stirrer and was magnetically agitated at 75 rpm [20]. Samples of 1 ml were withdrawn from the beaker at specified time intervals over a period of 24 h and were replaced by equal volume of fresh SWF. The amount of drug released with respect to time was determined by UV-Vis spectrophotometer at 290 nm against a standard curve of embelin in

simulated wound fluid in the concentration range of 1 to 10 $\mu\text{g/ml}$. The study was performed in six replicates.

In vivo studies

In vivo studies on rats were carried out after to evaluate the wound healing activity of the optimized hydrogel formulation. Study was started after getting the approval from Institutional Animal Ethical committee (Approval No: PERD/IAEC/2010/001). Six male Sprague–Dawley rats, weighing approximately 200–300 g, were procured from animal house of PERD center, Ahmedabad. The rats were housed in the animal house and were kept in standard sized metallic cages (3 rats/cage) in a well-ventilated room, and at a temperature of about 26–28 °C. They were acclimatized to the animal house condition for 7 days during which they were allowed to free access of water and food. The dorsal abdominal area of the animals was shaved in the direction of tail to head. Animals were anesthetized using diethyl ether and four excision wounds of full thickness, each 1.0 cm (circular area = 78.5 mm²) in diameter and 0.2 mm in depth along the marked area using a surgical blade, were created on each animal. The area for excision was marked using a marker. The wounds were cleaned with a sterile cotton prior to the reading and treatments.

The excised wounds were covered with the optimized hydrogel dressings containing embelin and were fixed with elastic adhesive tape (Medi grip). Moisturex[®] cream (Ranbaxy) and a blank hydrogel sheet (without embelin) were used as standard and control samples, respectively.



Moisturex cream was chosen as standard sample for its reported wound healing activity (<https://www.mims.com/india/drug/info/moisturex/moisturex%20cream>).

Another reason of this selection was due to non-availability of commercial medicated hydrogel sheet.

Dressings were changed every day. The wound healing rate was measured at the 3rd, 6th, 9th, 12th, and 15th day post-operation in terms of percent wound contraction [21]. The area of the wound was measured by tracing the size of the wound on a butter paper, and then the area was measured using a graph paper. The grouping of the wounds on the animals was done randomly into four groups: a, normal control (treated with saline); b, hydrogel with drug (embelin); c, hydrogel without drug and d: marketed preparation (Moisturex® cream). The dressing on the wound was shifted from animal to animal in a clockwise direction on the back of the rat as a, b, c, d; d, a, b, c; c, d, a, b, and likewise; this shifting of the dressing was done in order to avoid any biasness in the position of the wounds which might affect the healing process. The wound contraction was measured by the formula mentioned below:

$$\% \text{wound contraction} = \left(\frac{A_0 - A_t}{A_0} \right) \times 100 \quad (4)$$

where A_0 is wound size (area) at the initial time and A_t is wound size (area) at time t .

After the experiment, the animals were killed by cervical dislocation method followed by sacrifice, and the carcasses were incinerated.

Results and discussion

The purity and identity of the isolated embelin were compared against the reference standard. The crystallization of the embelin from *E. ribes* yielded 97% purity determined by HPLC. The UV-Vis spectral overlap of sample and reference peaks at the start, middle, and end of peaks were compared by HPTLC (Fig. 2). The UV spectra showed an absorption maximum at 289 nm which was found to be similar to reference standard (λ_{max} , 290 nm). The melting point was found to be 142–144 °C against the reported value of 142–143 °C, and the mass spectra of isolated compound showed (M-1) m/z ratio of 293.4 against the reported molecular weight of embelin 294.5. Thus, the isolated compound was confirmed as embelin and the same was used for further experimentation.

The hydrogel sheets using different compositions of PVA and PEG 400 (Table 1) were prepared by freeze-thaw cross linking after three cycles and were subjected to the various evaluation parameters as mentioned in the methodology section. The gel fraction of the prepared hydrogels was found to be in the range of 55–95% (Fig. 3). The gel fraction provides the extent to which

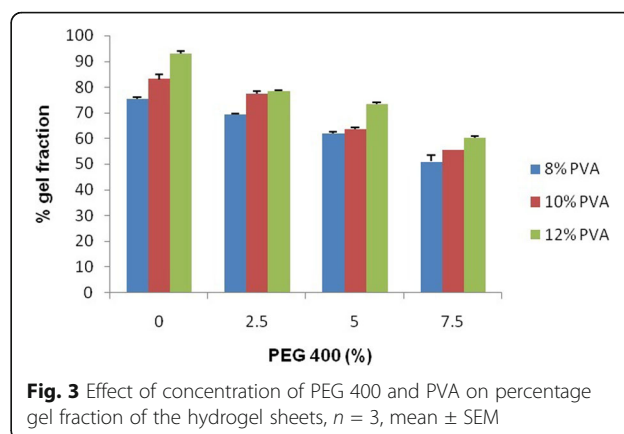


Fig. 3 Effect of concentration of PEG 400 and PVA on percentage gel fraction of the hydrogel sheets, $n = 3$, mean \pm SEM

the polymer chains have cross-linked with each other. In other words, the more the gel fraction the more is its crosslinking ability, and thus the more is its mechanical strength. It was found that as the concentration of PVA increased, the gel fraction of the sheet hydrogels also increased proportionately. This may be attributed to the increased crosslinking of polymer chains with the increasing concentration of polymer. The addition of PEG within the formulation decreased the percent gel fraction of the sheet hydrogel suggesting that it is somewhere interfering with the cross linking of PVA, and thereby decreasing the gel fraction.

The swelling index of the hydrogels was found to be a maximum of 372% as shown in Fig. 4. The swelling of a sheet hydrogels becomes very important in case of heavily exuding wounds. This helps in preventing the accumulation of the wound fluid in the wound bed, and thereby decreasing the chances of any bacterial contamination [22, 23]. It is observed from the graph that as the concentration of PVA increased, % swelling ratio also decreased. As an explanation, the higher concentration of polymer increases the cross linking, and thereby decreasing the porosity within the gel structure which results in a decrease in % swelling ratio. Addition of PEG

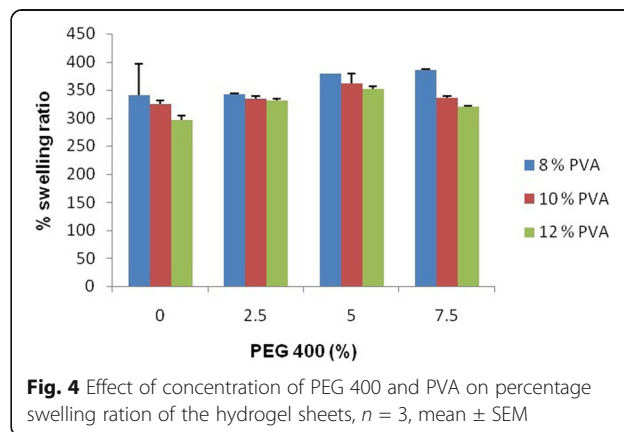
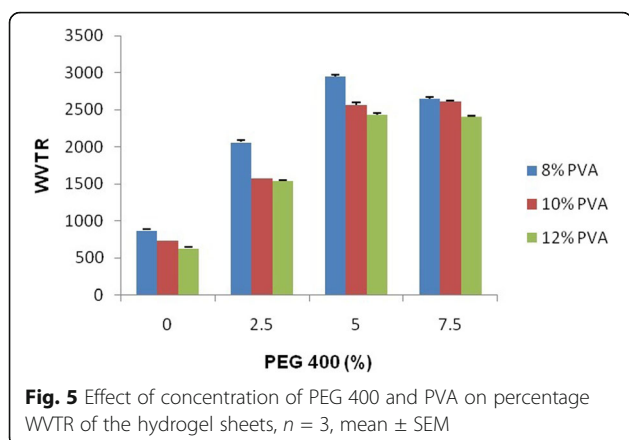


Fig. 4 Effect of concentration of PEG 400 and PVA on percentage swelling ratio of the hydrogel sheets, $n = 3$, mean \pm SEM



400 to the PVA solution was found to increase the % swelling ratio up to 5% w/w concentration of PEG 400. As explained in the gel fraction study, PEG 400 hinders the process of polymer cross-linking, produces a lesser cross-linked hydrogel matrix as compared to the PVA alone and makes the hydrogel sheets more porous as compared to only PVA hydrogel sheets.

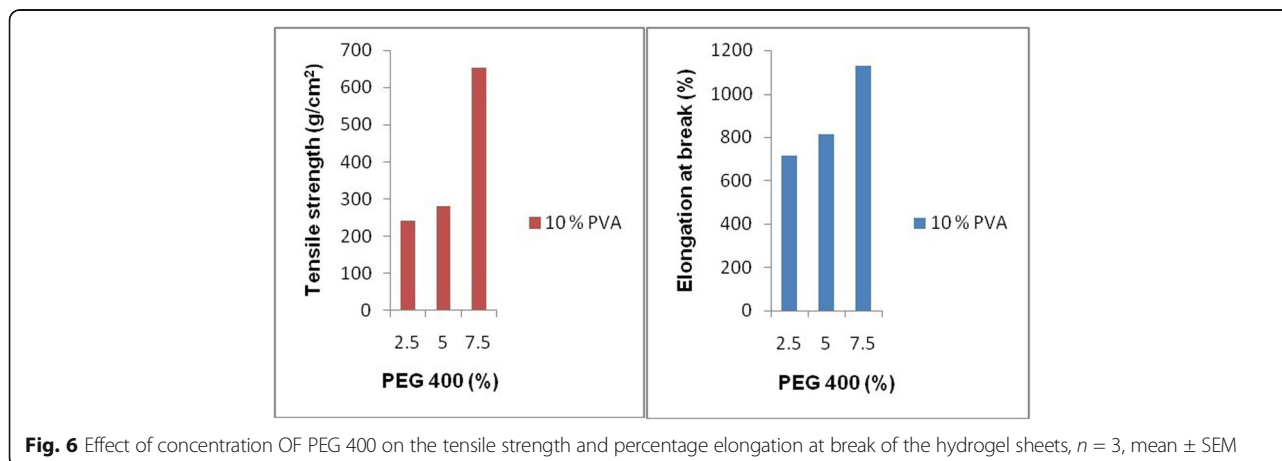
The WVTR of the various formulations prepared by the F-T method using PEG 400 is shown in Fig. 5. The WVTR of the hydrogel sheets containing only PVA is very less as compared to the hydrogel sheets prepared by PVA and PEG 400. PEG 400 as an interference to PVA cross-linking helps to maintain porosity within the hydrogel sheets up to a certain concentration. The hydrogels are the cross-linked polymers. Thus, the extent of its cross-linking determines its ability to transmit the vapor or the air to and fro from the hydrogel. The WVTR plays an important role to keep the wound bed moist. If the WVTR of the hydrogel is more, it makes the wound bed very moist, whereas low WVTR makes the wound bed dry. Thus, a hydrogel dressing should

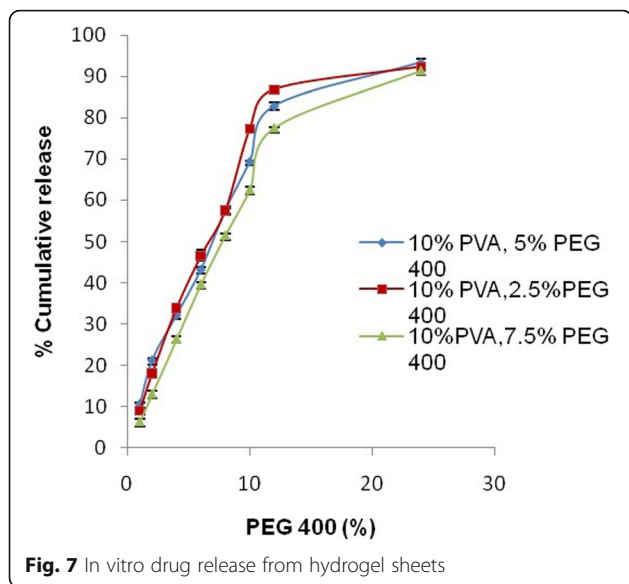
have an optimum WVTR, so that it can provide proper moistening to the wound bed, and thus helps in enhancing the healing process. The WVTR of an ideal wound dressing is reported to be 2000–2500 g/m² day [16], our hydrogel sheets (formulation IV to IX) also fell under that range [24].

From the above three parameters, it was found that the hydrogels containing 10% PVA irrespective of the PEG 400 concentration, had a desirable gel fraction (63%), swelling ratio (318.6%), and WVTR (2503 g/m² day). So, then to decide the optimum composition of PEG 400, the hydrogel sheets with 10% PVA at 2.5, 5, 7.5% w/w concentration of PEG 400 were further tested for their tensile strength. Figure 6 shows the mechanical strength of the hydrogel sheets in terms of their tensile strength and percentage elongation at break point. It was found that with the increase of PEG 400 concentration, tensile strength and the elongation at break of the hydrogel sheets also increased. It suggests that the incorporation of PEG 400 makes the hydrogel sheets tougher and more elastic. The hydrogel sheets containing 10% w/w PVA, 5% w/w PEG 400, and 0.2% w/w embelin resulted an optimum tensile strength (> 250 g/cm²) and percentage elongation at break point (> 800%), and thus was chosen for the further studies.

The in vitro release studies were performed on three compositions of hydrogel sheets containing 10% PVA each with three different composition of PEG 400 (2.5, 5, and 7.5% w/w). Figure 7 represents the release profile of embelin from the hydrogel sheets. Other than the hydrogel sheet with 10% PVA and 7.5% PEG 400, the remaining two formulations produced more than 80% drug release at 12 h. However, all of them showed about 90% of embelin release at the end of 24 h. The release followed a linear pattern and was found to follow zero order kinetics up to 12 h.

Figure 8 represents the surface morphology of the optimized hydrogel formulation before and after the





dissolution studies up to 24 h. It is observed that Fig. 8b (after dissolution) shows more porous nature as compared to Fig. 8a (before dissolution). Being soluble in nature, PEG 400 helps in channel formation within the hydrogel sheets during dissolution and embelin is released into the dissolution medium which ultimately makes gel matrix porous in nature.

The estimation of in vivo efficacy of the developed hydrogel was performed using the full-thickness excision wound model. The optimized hydrogel sheet with a composition of 10% PVA, 5% PEG 400, and 0.2% embelin was used for this study. Figure 9 shows the photographs of the wounds treated with the various dressings and the control wounds (untreated). The results obtained on the 3rd day post-treatment showed 30–40% reduction in wound size

(Fig. 10) in all the animals. However, it was noticed that the wounds treated with the medicated hydrogel showed a maximum reduction in wound area as compared to other groups. It was also observed that the swelling around the wound area decreased in all the animals on the 3rd day after the initiation of the treatment. As expected, the wounds treated with drug loaded hydrogels showed no sign of dry scar formation, rather the wounds were moist, which justify the better wound healing of that treatment as compared to the control and marketed formulation. At the end of 6th day post-treatment, the hydrogel treated wounds showed a formation of a thin epidermis without any scar infection. This formation of epidermis was only found in the wounds treated with hydrogels which can be attributed to the property of the hydrogel to maintain a moist environment in the wound region. At the end of 9th day post-treatment, all the wounds in group “b” (medicated hydrogel) showed 90% reduction in wound area and the epidermis layer showed thickening. The control and marketed groups also showed a reduction in wound area but the epidermis was not completely formed. It is mentioned in the literature [23] that during the initial phases of healing one needs a constant flow of the growth factors and nutrients in the wound area so as to initiate the epithelization and enhance the formation of granulating tissue [24]. The wound healing process needs an initial early re-epithelialization and granulation tissue formation that was confirmed by our work with the embelin containing optimized hydrogel sheet. This hydrogel sheet was found to be very much useful for the rapid initialization of wound healing process.

The observed results suggest that the hydrogels help in enhancing the healing process by creating a moist environment. It is observed from the results that at the end of 12th and 15th days post-treatment, all the treated groups showed a similar contraction of the wound area.

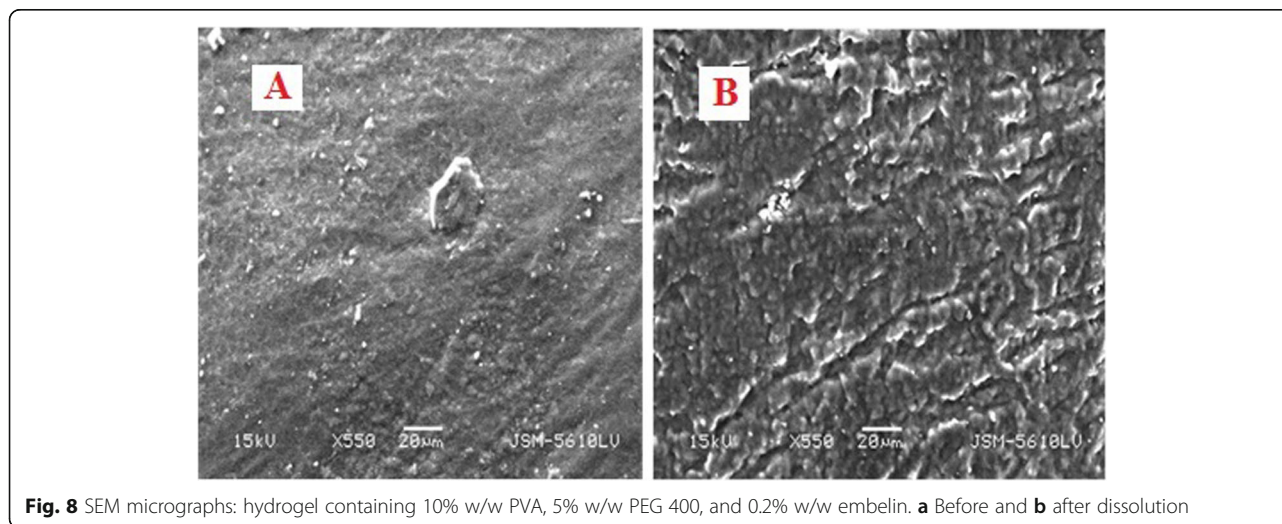




Fig. 9 Photographs of wound treated with (a) sterile gauze, (b) hydrogel with 0.2% embelin, (c) hydrogel without any drug, and (d) marketed product at a 0 days, b 3 days, c 6 days, d 9 days, e 12 days, and f 15 days of post-operation

However, the appearance of the wounds treated by the medicated hydrogel (group b) were uniform, cleaner, and healthy as compared to all the other groups (a, c, and d). Thus, we can infer from these results that medicated hydrogels are good for initial wound healing process as they initiate the healing process faster.

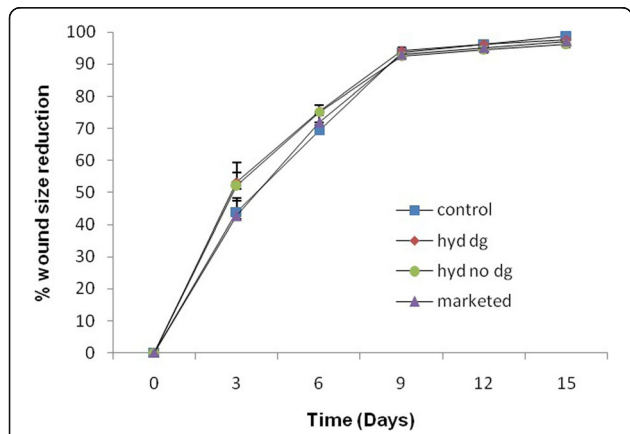


Fig. 10 Percentage wound size reduction of the control, hydrogel with drug, hydrogel without drug, and marketed product, $n = 6, p < 0.05$

Day-wise comparative healing for medicated hydrogel sheet against standard or control formulation is summarized in Table 2. In summary, it was found that wound healing is a complex process and the key points through healing rate is increased by providing an environment suitable for healing in the initial stages itself. It is known that wound repair must occur in a physiologic environment which is conducive to tissue repair and regeneration. In addition, increased metabolic demands are made by the inflammation and cellular activity in the healing wound, which may require increased protein or amino acids, vitamins, and minerals. Thus, due to all these reasons, one needs to have a moist environment in the initial stages of wound healing so that the migration of all the aforesaid factors can be available more and enhance the wound healing. The main objective in wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort, and scarring to the patient which is provided by the prepared hydrogel. Superior activity of the embelin containing hydrogels can be attributed to the wound healing activity of the drug itself and its established antimicrobial activity [12, 13].

Table 2 Day-wise comparative healing for medicated hydrogel sheet against standard/control formulation

Observation day	Standard (Moisturex® cream)/control (blank hydrogel sheet)	Test (medicated hydrogel sheet)
3rd day post treatment	30–40% reduction in wound size and presence of dry scar.	30–40% reduction in wound size. Additionally, absence of dry scar formation, rather the wounds were moist.
6th day post treatment	Absence of epidermis formation.	Formation of a thin epidermis without any scar formation.
9th day post treatment	90% reduction in wound area, but incomplete epidermis formation.	90% reduction in wound area with presence of thick epidermis layer.
12th and 15th day post treatment	Full contraction of the wound area but appearance of non-uniform new skin.	Full contraction of the wound area with appearance of new skin that was uniform, cleaner and healthy.

Conclusion

An optimized medicated hydrogel containing 0.2% embelin (isolated, purified, and characterized) was formulated with 10% PVA and 5% PEG 400. The optimized formulation was further characterized by the various physico-chemical parameters like swelling index, gel fraction, water vapor transmission rate, and mechanical strength. The *in vitro* release studies of the optimized formulation followed by zero order drug release kinetics with more than 80% embelin release up to 12 h. The *in vivo* studies on the rats showed that the optimized embelin-loaded hydrogel is the best for initial wound healing process as it initiates the healing process faster. These encouraging results should be further explored in large scale for confirmation of *in vitro* parameters as well as in healthy human volunteers to prove its *in vivo* efficacy.

Abbreviations

UV/VIS spectroscopy: Ultraviolet/visible spectroscopy; HPTLC: High-performance thin layer chromatography; HPLC: High-performance liquid chromatography; WWTR: Water vapor transmission rate; SEM: Scanning electron microscopy; PEG: Polyethylene glycol; PVA: Polyvinyl alcohol; SWF: Simulated wound fluid

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Authors' contributions

NS and UKM conceptualized the idea about the research work, guided the whole work, and wrote the research article. HS performed all experimental works. UKM was responsible for designing the formulation work. *In vivo* study was carried out under the supervision of MN. NS was responsible for data analysis. All authors read and approved the manuscript.

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Availability of data and materials

Authors agree to provide additional data and raw materials available with us to the Journal if required.

Ethics approval and consent to participate

In vivo efficacy of the developed formulation was on Sprague–Dawley rats. Study was started after getting the approval from Institutional Animal Ethical committee (Approval No: PERD/IAEC/2010/001).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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