

Original Research Article

Formulation, characterization and wound-healing potential of emulgel and in-situ gel containing root extract of *Saussurea lappa* Clarke (Asteraceae)

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Abstract

Purpose: To investigate the wound-healing potential of herbal formulations (emulgels and in situ gels) containing *Saussurea lappa* root extract (SLRE) via excision wound induction in albino rats.

Methods: Preliminary phytochemical analysis of the methanol extract of roots of *Saussurea lappa* (SLRE) was performed using standard procedures. In vitro anti-inflammatory assay of SLRE was conducted using heat-induced hemolysis method at a concentration of 100 µg/mL. Acute toxicity of SLRE was also evaluated in mice at a single dose of 1000 mg/kg for 24 h. Emulgels and in situ gels were prepared using different concentrations of SLRE and assessed for their organoleptic and physical properties. In vitro drug release studies of the prepared formulations were carried out by Franz diffusion cell and the data fitted into various pharmacokinetic models. Wound healing was assessed using excision wound induction (380 mm²) on dorsal surface of male albino rats. Each formulation (F4, F5, F6, G1, G2 and G3) and pyodine gel (standard) were applied topically (0.5 g) for 20 days. Wound contraction was measured every fourth day.

Results: SLRE showed 42.8 % inhibition in heat-induced hemolysis on erythrocyte membrane model, compared to aspirin (positive control). Moreover, SLRE did not cause mortality in mice at the given doses. All the formulations were stable after one month stability check at 40 °C for emulgels and at 25 °C for in situ gels. All the formulations followed first order drug release pattern. In situ gel (G3) exhibited better wound healing (100 ± 0.0028) than emulgel (F6, 99 ± 0.004) containing 5 g extract and standard pyodine gel (91 ± 0.014 , $p < 0.05$).

Conclusion: The results indicate that in situ gel of SLRE exhibits significant wound healing in rats. Thus, the findings present a strategy for the formulation of gel products with better wound healing potentials.

Keywords: *Saussurea lappa*, Wound healing, Emulgel, In situ gel, Herbal formulation

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INTRODUCTION

Natural products are very diverse and almost 35,000 - 70,000 plants species have been

screened till date for their pharmacological activities. Ethno-pharmacological use of crude drugs provided a major clue in drug discovery. Data suggested that more than 50 % of medicines used during last 30 years were of

natural origin [1]. *Saussurea lappa* Clarke belonging to Asteraceae family of flowering plants and commonly known as costus or kuth occurs in South East Asia and Pakistan. This plant is traditionally used as home remedy in wide range of ailments including myalgia, cancer, rheumatoid arthritis and wound healing, etc [2].

Healing of wound is an important physiological mechanism that involves hemostasis, inflammation, proliferation and remodeling with highly programmed phases. These phases are necessary for proper wound healing in a proper sequence and time frame [3]. *Saussurea lappa* roots contain variety of phytochemicals like sesquiterpene-lactones that have anti-inflammatory and wound healing potential. Alnahdi et al. reported that gastric ulcers were inhibited by costunolide and saussureamines which were obtained from roots of *Saussurea lappa* [4]. Cyanopicrin is another phytochemical found in roots of *Saussurea lappa* that has been reported as potent immunosuppressive agent [5].

Topical drug delivery system is a conventional drug delivery system in which drugs are easily delivered through skin and include ointments, creams etc. But topical dosage forms have some drawbacks like low bioavailability and poor retention [6]. Emulgels and *in situ* gels are emerging techniques for topical drug delivery system. Emulgels provide dual control release in the form of emulsion as well as gel [7] whereas *in situ* gelling system involves use of polymers that have phase transition from solution to gel upon alterations in physico-chemical properties of drug [8]. Herbal formulations have become popular due to their natural origin and are used in variety of health ailments like liver problems, diabetes and heart problems [9]. Therefore, main purpose of the present study is to develop herbal formulations of *Saussurea lappa* root extract and evaluate their wound healing potential.

EXPERIMENTAL

Chemicals

Chloroform, n-hexane, ethyl acetate and methanol were used as solvents for extraction. HPMC k15M, polaxomer P407, carbopol 934, polyvinyl alcohol and benzyl alcohol were used as polymers for formulations development. Aspirin was obtained as a gift from Medizan Laboratories Pvt. Ltd. Islamabad and used as standard for *in vitro* anti-inflammatory assay. Pyodine gel was purchased from local Pharmacy and used as positive control for wound healing

activity. All the chemicals were of highest grade purity.

Plant collection and extraction

The roots of *Saussurea lappa* were collected from the wild cultures growing in and around Swat valley, Pakistan in April 2018. The plant had been identified by Dr. Mushtaq Ahmed, a taxonomist at the Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan and provided voucher specimen number as 025120. Roots were shade dried and then grounded coarsely. Four extracts were prepared using methanol (MeOH), ethyl acetate (EtOAc), chloroform (CF) and n-hexane through soxhlet extractor which were concentrated under reduced pressure at 40 - 50 °C using rotary evaporator.

Thin layer chromatography and preliminary phytochemical screening

Analytical TLC was performed for the detection of sesquiterpene lactones in the crude extracts. The plates (TLC Silicagel; Merck KGaA, Darmstadt, Germany) were spotted with 5 - 10 µg of each extract and placed in glass chamber containing chloroform and methanol (9:1). After development, all the plates were dried and exposed to iodine vapors followed by heating. After 20 minutes, the plates were observed. Furthermore, preliminary phytochemical analysis of the extracts was performed to detect the presence of flavonoids, terpenoids, carbohydrates, alkaloids, proteins, tannins, saponins, phenolic compounds and glycosides using standard procedures [10].

In vitro anti-inflammatory assessment of crude extract

The blood sample (5ml) was withdrawn from a healthy human volunteer and centrifuged for 10 min at 3000 rpm. After washing the blood cells with normal saline thrice, finally 10 % v/v suspension of the RBCs was prepared by adding normal saline. For heat induced hemolytic assay 2ml of mixture containing 1 mL of 10 % RBCs suspension and 1 mL test sample (100 µg/mL of crude extract) were mixed. For positive control aspirin while for negative control normal saline was used. The mixture was incubated in a water bath at 56 °C for 30 min and then centrifuged again at 2500 rpm for 5 min. Absorbance of the supernatants was recorded at 560 nm [11]. The percentage inhibition (H) of hemolysis was calculated as in Eq 1.

$$H (\%) = \{(Ac - As)/Ac\}100 \dots\dots\dots (1)$$

where A_c and A_s are the absorbance of control and test samples respectively.

Animals

Male Balb-C mice (20 - 30 g) and Albino rats (180 - 220 g) were housed at the Animal House of Riphah Institute of Pharmaceutical Sciences Islamabad, at 25 ± 2 °C. All the experiments complied to the rules of Institute of Laboratory Animals Resources, Commission on Life Science University, National Research Council, 1996 [12] and approved by Research and Ethics Committee (REC) of Riphah Institute of Pharmaceutical Sciences Islamabad (approval ref no. REC/RIPS/2019/19).

Acute toxicity study

Acute oral toxicity assay was performed on twelve male Balb-C mice (20 – 30 g) which were divided into two groups (n = 6). Experimental group was orally given 1000 mg/kg dose of SLRE dissolved in normal saline while mice in control group were administered normal saline (10 mL/kg). Any sign of toxicity or death was observed during 24 h [13].

Formulation development

Emulgels

Six formulations of emulgels (F1-F6) containing SLRE (0.5-5 g) were prepared by separately making oil phase (liquid paraffin and span 80) and aqueous phase (distilled water and tween 80) Oil phase was added in aqueous phase and

carbopol 940 dissolved in distilled water was added. After that methanolic extract, PEG 4000, methyl paraben and eucalyptus oil were incorporated in this mixture and blended thoroughly to make final formulation [14]. Composition of emulgels is shown in Table 1.

In situ gels

Three formulations of *in situ* gels (G1-G3) with SLRE (3-5 g) were prepared by mixing aqueous preparation of 1.5 % polaxomer 407, 2 % carbopol 940, 15 % hydroxy propyl methyl cellulose (k15) and 1 % poly vinyl alcohol. Lyophilized extract was added in formulations and mixed thoroughly [15,16]. Composition of *in situ* gels is shown in Table 2.

Characterization of formulations

Emulgels were observed for appearance, grittiness and phase separation. Phase separation was checked through centrifugation of emulgels at 3000 rpm for 10 min. Spreadability was determined by placing 350 mg of formulation on a glass slide while second slide was placed from the 5 cm over the first slide and diameter of circle was measured. A pH meter was used to determine pH while Brookfield viscometer (spindle 61 at 100 rpm) was used to measure viscosity. Swelling index was noted by placing 1 g of formulation on aluminum foil in which pores were created and then placed in 10 mL of 0.1N NaOH. Samples were collected and dried for 30 min. After drying samples were reweighed and % swelling index (SW) was calculated as shown in Eq 2.

Table 1: Composition of emulgel formulations containing SLRE

Formulation	SLRE (g)	Carbopol 940 (g)	Span 80 (mL)	Tween 80 (mL)	Liquid Paraffin (mL)	Methyl paraben (g)	Eucalyptus oil (mL)	PEG 4000 (mL)	Water (mL)
F1	0.5	3	1.8	2	10	0.1	0.1	10	10
F2	1	3	1.8	2	10	0.1	0.1	10	10
F3	2	3	1.8	2	10	0.1	0.1	10	10
F4	3	3	1.8	2	10	0.1	0.1	10	10
F5	4	3	1.8	2	10	0.1	0.1	10	10
F6	5	3	1.8	2	10	0.1	0.1	10	10

Table 2: Composition of *in situ* gel formulations containing SLRE

Formulation	S. lappa extract (g)	Carbopol 940 (g)	HPMC k 15 (g)	Poly vinyl alcohol (g)	Polaxomer 407 (g)	Distilled water (mL)
G1	3	1	7.5	0.5	7.5	200
G2	4	1	7.5	0.5	7.5	200
G3	5	1	7.5	0.5	7.5	200

$$SW = \{(W_t - W_0)/W_0\}100 \dots\dots\dots (2)$$

While W_t is the weight after time t and W_0 is the weight at time zero respectively.

In situ gels were observed for appearance while pH, spreadability and viscosity were determined as mentioned earlier for emulgels [17]. Moreover, Emulgels were stored at 40 °C whereas *in situ* gels were stored at 25 °C and different parameters like pH, viscosity were determined every week for one month according to ICH guidelines [18].

***In vitro* drug release studies**

Modified Franz diffusion cell was used for *in vitro* drug release studies using 0.22µm dialysis membranes. Donor compartment was filled with emulgels and *in situ* gels whereas recipient compartment contained phosphate buffer (pH 6.0). Magnetic bar with teflon coating was put in recipient chamber and rotated at 50 rpm. Temperature of Franz diffusion cell was kept at 37 °C by circulating heated water in the outer jacket. Samples were removed at 0, 1, 2, 3, 4, 5, 6, 7 and 8 h while absorbance was determined at 332nm using UV-VIS spectrophotometer as shown in Eq 3 after dilution with phosphate buffer [14].

$$A = \{(A_s / A_c)\}100 \dots\dots\dots (3)$$

Where A_c and A_s are the absorbance of control and test sample respectively.

Evaluation of kinetic release mechanism

Data obtained from *in vitro* drug release study was fitted into various kinetic models like first order model, Higuchi model and Hixson Crowell model for the evaluation of kinetic release [19].

First order model

It establishes the relationship between percentage drug release and time. Integration and rearrangement is shown in Eq 4.

$$\text{Log } C_t = \text{Log } C_0 + Kt/2.303 \dots\dots\dots (4)$$

where K is the first order rate equation, C_0 is the initial concentration of the drug, C_t is the remaining drug at any particular time, and t is the time in hours.

Higuchi model

It is most prominent pharmacokinetic model that involves drug dissolution and diffusion which

depends on drug concentration. Simplified form of Higuchi equation is shown in Eq 5.

$$Q = K_H \times t^{1/2} \dots\dots\dots (5)$$

where Q is the Cumulative amount of drug released at time t , K_H is the Higuchi release rate constant and $t^{1/2}$ is the square root of time.

Hixson-Crowell model

It mainly describes the drug release from a polymeric system that involves changes in surface area and diameter of drug molecules. Simplified relationship between drug release and time is given in Eq 6.

$$W_0^{1/3} - W_t^{1/3} = K_{Hc} t \dots\dots\dots (6)$$

where $W_0^{1/3}$ is the cube root of initial amount of drug present in the matrix, $W_t^{1/3}$ is the cube root of remaining amount of drug in matrix at time, K_{Hc} is the release rate constant and t is the time.

Wound healing study

Male albino rats in nine (I-IX) groups (n=4) were used for the study. Ketamine (25 mg/kg) was used for anesthesia and the dorsal surface of each rat was shaved with sterile razor. Excision wound of 380 mm² was induced in all rats. The wounds were kept open and treated as follows:

Groups I-VI were treated topically with 0.5g of each formulation (F4, F5, F6, G1, G2 and G3) while group VII served as positive control group administered with topical pyodine gel (PG).

Group VIII was treated with crude extract SLRE (200 mg/10mL) while Group IX was untreated group and served as negative control group.

Wound size was measured after every four days for a period of twenty days and percentage wound contraction (WC) was determined as mentioned in Eq 7 [20].

$$WC (\%) = \{(W_0 - W_t)/W_0\}100 \dots\dots\dots (7)$$

where W_0 is the initial wound size and W_t is the wound size on specific day.

Statistical analysis

Values were presented as mean ± standard error of mean (SEM) and were analyzed by one-way analysis of variance (ANOVA) in Microsoft Excel[®]. Differences in values were considered statistically significant at $P < 0.05$.

RESULTS

Thin layer chromatography and preliminary phytochemical profile

Brownish spots of sesquiterpene lactones were detected on the TLC plate containing methanolic extract of *Saussurea lappa* roots (SLRE) whereas no spots were observed in plates containing EtoAc, CF and n-hexane extracts. Preliminary phytochemical screening of SLRE showed positive results for alkaloids, glycosides, flavanoids, terpenoids, saponins, tannins, phenols and carbohydrates but negative results for proteins. These results are presented in Table 3.

Table 3: Preliminary phytochemical screening of *S. lappa* root extract

Phytochemical	Indication	Result
Alkaloids	Reddish brown precipitates	+
Glycosides	Brown color	+
Saponins	Stable froth for 10 minutes	++
Phenols and tannins	Blue green color	++
Proteins	No change	-
Carbohydrates	Brick red color	+
Flavanoids	Intense yellow color	++
Terpenoids	Gray color	++

(++)Strongly Present, (+)Weakly Present, (-) Absent

Table 4: Some characteristics of emulgels

Formulation	pH	Viscosity (cps)	Swelling index (%)	Spreadability (cm/s)
F1	6.83± 0.021	1567.8 ± 0.031	56.2 ± 0.032	18 ± 0.041
F2	6.92 ± 0.028	1575.0 ± 0.042	69.3 ± 0.021	20 ± 0.045
F3	6.35± 0.027	1589.0 ± 0.032	88.5 ± 0.042	16 ± 0.039
F4	6.79± 0.031	1596.0 ± 0.021	85.4 ± 0.041	16 ± 0.023
F5	7.12± 0.025	1612.3 ± 0.026	89.6 ± 0.038	18 ± 0.039
F6	7.33±0.026	1619.0 ± 0.028	97.6 ± 0.024	20 ± 0.038

Values are mean ± SEM (n = 4) P < 0.05

Table 5: Characteristics of *in-situ* gels

Formulation	pH	Viscosity (cps)	Spreadability (cm/s)
G1	6.73±0.014	1665.0±0.021	17±0.012
G2	6.62±0.015	1682.5±0.034	19±0.017
G3	6.89±0.021	1696.0±0.023	16±0.018

Values are mean ± SEM (n = 4, p < 0.05)

In vitro anti-inflammatory activity

SLRE exhibited 42.8 % inhibition in heat induced hemolysis on erythrocyte membrane model compared to aspirin (51 %) at a concentration of 100 µg/mL.

Acute toxicity

Neither mortality nor any sign of toxicity at tested dose (1000 mg/kg) was observed after 24 hr.

Characteristics of the formulations

Emulgel formulations (F1-F6) were yellowish brown in color while *in situ* gels (G1-G3) were brown in color. No grittiness and phase separation (done by centrifugation) was observed in any formulation. pH and viscosity of emulgels and *in situ* gels did not change. Results for characterization of emulgels are shown in Table 4 while *in situ* gel formulations are presented in Table 5. Furthermore, F6 and G3 were placed at accelerated conditions to check their stability because profound wound healing effect was observed by these two formulations. Stability studies for emulgels were performed at 40 °C whereas *in situ* gels were evaluated by storing them at 25 °C for 4 weeks as shown in Table 6 and 7 respectively. No change in color was seen and phase separation was also not observed throughout the study period.

Drug release kinetics

Various mathematical models were designed to correlate drug permeation profile with drug

Table 6: Stability of emulgel (F6) at 40 °C

Time (week)	Parameter	
	pH	Viscosity (cps)
0	6.89±0.031	1619.7±0.048
1	6.88±0.021	1600.7±0.054
2	6.88±0.033	1584.1±0.056
3	6.89±0.021	1578.2±0.096
4	6.89±0.034	1570.8±0.052

Values are mean ± SEM P < 0.05

Table 7: Stability of *in situ* gel (G3) at 25 °C

Time (week)	Parameter	
	pH	Viscosity (cps)
0	6.89±0.051	1696.0±0.068
1	6.89±0.073	1698.9±0.074
2	6.89±0.081	1698.5±0.059
3	6.90±0.093	1699.5±0.088
4	6.90±0.096	1699.6±0.093

Values are mean ± SEM (p < 0.05)

release kinetics [19]. The findings showed that all formulations released drug constantly over the period of time and better release rates were observed with *in situ* gels when compared to emulgels. All formulations followed first order release pattern. Values of r^2 and r^3 are presented in Tables 8, 9 and 10.

Table 8: First order release pattern of emulgel (F6) and *in-situ* gel (G3)

Time (h)	Log cumulative percent release (r^2)	
	Emulgel	<i>In situ</i> gel
1	2.63	2.19
2	1.66	1.58
3	1.20	1.26
4	0.94	1.01
5	0.83	0.84
6	0.72	0.72
7	0.62	0.63
8	0.55	0.56

Table 9: Higuchi model for release pattern of emulgel (F6) and *in-situ* gel (G3)

Square root of time ($t^{1/2}$)	Cumulative percent release (r^2)	
	Emulgel	<i>In situ</i> gel
1	15	10.23
1.41	20.56	17.73
1.73	21.96	26.98
2.01	22.56	30.21
2.22	29.14	30.96
2.44	32.34	31.56
2.64	31.46	32.63
2.82	31.78	33.89

Wound healing activity

All the emulgels (F1-F6) and *in situ* gels (G1-G3) were tested for wound healing potential but

emulgel formulations F1, F2 and F3 showed negligible wound healing effect. So we did not use these formulations further. The emulgels (F4-F6) and *in situ* gel (G1-G3) demonstrated substantial contraction of wounds and improved epithelialization in rats as compared to control groups. Results are shown in Table 11.

Table 10: Hixson-Crowell model for release pattern of emulgel (F6) and *in-situ* gel (G3)

Time (h)	Cube root of release (r^3)	
	Emulgels	<i>In situ</i> gels
1	-2.46	-2.19
2	-0.03	-0.38
3	-0.09	-0.26
4	-0.04	-0.09
5	-0.09	-0.03
6	-0.04	-0.02
7	-0.02	-0.01
8	-0.01	-0.02

DISCUSSION

This study demonstrated the wound healing potential of herbal formulations (emulgels and *in situ* gels) of SLRE.

Emulgels and *In-situ* gelling system are the recent formulations in novel drug delivery systems that provide controlled drug release [6,7]. *Saussurea lappa* possess important pharmacological properties such as anti-ulcer, wound healing, anti-inflammatory and anti-convulsant, etc [21].

The methanolic extract of *Saussurea lappa* was shown to contain alkaloids, glycosides, flavonoids, terpenoids, carbohydrates, saponins and tannins [10]. Sesquiterpene lactones were also detected in methanolic SLRE through TLC therefore, formulations were developed using methanolic extract. Acute toxicity study of SLRE indicates lower toxicity profile of *Saussurea lappa*. *In vitro* anti-inflammatory assay showed that SLRE could be beneficial in reducing inflammation because it had inhibited heat induced hemolysis and shown comparable effect with aspirin in this study. As NSAIDs cause gastric ulcers over long term use while the herbal extracts are safer than these drugs. Herbs are always important in maintaining normal health due to their low toxicity profile and the use of herbal formulations is also becoming popular because of lesser side effects [9].

Emulgels (F1-F6) and *in situ* gels (G1-G3) were evaluated by organoleptic and physical parameters like color, grittiness, phase separation, spreadability, pH, swelling index and viscosity. No change in color of formulations was

Table 11: Wound healing assessment of formulations in experimental rats

Formulation	Wound contraction (%)				
	Day 4	Day 8	Day 12	Day 16	Day 20
F4	10 ± 0.014	35 ± 0.025	50 ± 0.030	67 ± 0.031	84 ± 0.036
F5	13 ± 0.022	37 ± 0.400	53 ± 0.018	72 ± 0.016	88 ± 0.027
F6	20 ± 0.017	43 ± 0.025	62 ± 0.026	79 ± 0.024	99 ± 0.004
G1	21 ± 0.010	46 ± 0.024	72 ± 0.025	87 ± 0.020	96 ± 0.008
G2	28 ± 0.064	50 ± 0.025	78 ± 0.021	92 ± 0.013	97 ± 0.008
G3	31 ± 0.009	55 ± 0.020	84 ± 0.019	99 ± 0.002	100 ± 0.002
PG	17 ± 0.009	41 ± 0.029	70 ± 0.020	84 ± 0.023	91 ± 0.014
SLRE	22 ± 0.013	43 ± 0.027	75 ± 0.026	86 ± 0.010	94 ± 0.019
Untreated group	9 ± 0.011	26 ± 0.017	45 ± 0.006	65 ± 0.021	77 ± 0.016

Values are mean ± SEM (n = 4, p < 0.05)

observed at the end of study indicating that formulations were stable at storage conditions. No grittiness felt in any formulation displaying that particle size of excipients is small and not increased with time. No phase separation on centrifugation in case of emulgels shows that formulations are stable. Viscosity of emulgel formulations decreased at high temperature because at high temperature surfactants in the emulgel can obtain energy from the heat and interfacial film between dispersed phase and dispersion medium become reduced and make the formulations less viscous.

While viscosity of *in situ* gels was increased because *in situ* gels undergo gelation on high temperature [14-15], the pH of the formulations did not change noticeably however slight variation was noticed in pH showing that formulations are stable and can be used topically for wound healing purposes without irritating the outer skin layers. Spreadability of all formulations was in an acceptable range revealing that formulations are easy to spread without any difficulty by little shear [17]. *In vitro* drug release results showed that formulations (F6 and G3) followed first order model for drug release which indicates that drug release is dependent on the quantity of drug left as compared to Higuchi model in which release of drug is dependent on diffusion of drug from polymers and Hixson Crowell model that describes percentage of drug remaining in matrix verses time [19].

Wound healing effect of formulations (F1-F3) was negligible which may be due to very low concentration of extract used. Formulations F6 and G3 exhibited maximum wound healing effect. It means that wound healing effect was dose dependent and results revealed that wound healing activity was improved as the amount of extract in the formulations increased. So these two formulations (F6 and G3) were selected for stability testing and *in vitro* drug release study.

Results of wound healing activity of emulgels and *in situ* gels were statistically significant in comparison to positive (PG) and negative control group (untreated group) with P<0.05. When emulgels and *in situ* gels results were compared, it was observed that *in situ* gels were more effective in healing the wounds due to their better *in vitro* drug release as polaxomer 407 inhibit the effect of efflux pumps that cause drug to stay into the cells for a longer period of time than emulgels and these results were statistically significant [21].

Moreover, wound healing results for SLRE were also statistically significant when compared to PG and untreated group P<0.05. The present study also indicated that *in situ* gel (G3) promoted better wound healing by increasing wound contraction and epithelialization. Emulgel containing SLRE also showed better wound healing as compared to pyodine gel may be due to the antioxidant rich compounds that are present in SLRE. Several studies have shown that *Saussurea lappa* is used in treatment of more than 43 diseases including wound healing [22]. Exact mechanism of wound healing shown by SLRE is still unknown therefore; further molecular studies are required to investigate the possible underlying mechanisms.

CONCLUSION

The findings of this study reveal that SLRE contains a variety of phytochemicals such as sesquiterpene lactones that are responsible for its *in vitro* and *in vivo* activities including inhibition of heat induced hemolysis and wound healing. *In situ* SLRE gel demonstrates higher wound healing properties than emulgel, SLRE and even pyodine gel. Further studies are required to determine its feasibility as an alternative herbal medicine for the management of wounds.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Ghulam Abbas Miana and Masood ur Rehman designed the study and supervised the project. Aitazaz Ahsan conducted literature search, experimental work, data analysis and drafted the manuscript. Kamil Anum and Imran Malik helped in carrying out the experimental work. Humaira Naureen supervised the study and helped in drafting and reviewing the final manuscript. All authors reviewed and approved the final manuscript for publication.

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