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FORMULATION DEVELOPMENT AND EVALUATION OF NOVEL HERBAL FILM FORMING SPRAY FROM *Glycyrrhiza glabra*

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INTRODUCTION

Human s existence on this earth has been made possible only because of the vital role played by the plant kingdom in sustaining life. The history of herbal medicines is as old as human civilization. **[Sundaresh I, 1973][Kokate.C.K. *et al.*, 2000],[Shastri.M.R, 1993]** Natural products (pure chemicals, extracts, and crude medications) can be of terrestrial or marine/aquatic origin and are obtained from higher plants, microorganisms, or animals. The therapeutic preparations made from these basic materials were typically made from a combination of different elements and took the form of crude drugs, such as dried herbs, or extracts thereof. Many of these supposedly therapeutic plants were subjected to chemical analysis with the introduction of European scientific techniques, which resulted in the isolation of active components. There has been constant work in this field since 1800 A.D., during which time several well-known medicinal plants were chemically examined and their active ingredients were identified. These substances, either in their pure form or as well-characterized extracts, were soon included in the pharmacopoeias of many nations after their isolation and characterization. **[Handa.S.S, 1991]**

More modern illnesses have become a menace to humankind. In spite of this, by 2000 A.D., the World Health Organization (WHO) had sworn to provide Health for all. Despite the enormous progress that modern medicine has achieved, there are still undiscovered treatments for conditions including AIDS, some types of cancer, arthritis, and Parkinsonism.

Remarkably, half of all prescription medications are either synthesised from natural models or directly drawn from natural sources as one of several constituents or as the only ingredient **[Mukaerjee. P. K, 2002] [Bhanu .P. *et al*, 2003)] [Gupta. A.K and Chitme., H.R, 2000]**.

The human body goes through a complicated and multifaceted process called wound healing in order to replace injured tissue and regain its structural and functional integrity. The body s amazing capacity for self-healing, from little cuts to large surgical incisions, is evidence of the complex biological processes at work. This thorough manual explores the many phases of wound healing, providing insight into the causal chain of events, cellular participants, and variables impacting the process **[Ahn C, Mulligan P. ,*et.al.* 2008.].**

The four separate but related stages of wound healing are hemostasis, inflammation, proliferation, and remodeling. **[Balaji S.M., *et al.*, 2008]. [Chan LK,*et.al.*,2006]. [Li.J., *et al.*, 2006].**

In recent pharmaceutical product development Film forming spray (FFS) found more useful for wound healing. A sprayed solution is used in the FFS medication delivery method. This technique uses the polymer as a matrix to generate a film when it comes into contact with the intended therapeutic location. [**Kate K, *et al.*,2017, Frederiksen K, *et al.*,2016, Ranade S *et al.*, 2017]** To regulate local or systemic effects, medication dosages in film-forming sprays also be able to change dependent upon the volume of the solution used in every spray. Additionally, an FFS distributes medications evenly and spreads effectively. Additionally, user-friendliness can boost patient adherence. **[Bakhshi A, *et al.*, 2008; Lu W, *et al.*, 2013]**

Licorice or Liquorice is the dried peeled or unpeeled roots and stolons of *Glycyrrhiza glabra* Linn, Family: *Fabaceae*. This plant has been used for its medicinal property for more than 4000 years. Licorice has proved to be effective in the treatment of disease like gastric ulcers, arthritis, allergy, inflammation, leukemia, cancer, psoriasis, atopic dermatitis and in hepato-toxicity**.[Deng S, *et al.* 2013]** Basically, licorice comprises of two components i.e. glycone and aglycone which are responsible for its medicinal properties. Glycone is glycyrrhizic acid (GA) and aglycone is glycyrrhetinic acid, Out of these, glycone part i.e. GA is an important compound responsible for the pharmacological and biological properties of licorice.

MATERIALS AND METHODS:

The roots of *Glycyrrhiza glabra* were collected and shade dried and powdered. Different oils extracts were extracted from different parts of plants and various preliminary phytochemical test

were performed to develop a formulation for an herbal film forming spray that can create a protective barrier over the wound site. To contribute to the development of a natural and alternative approach to wound care, providing a potential option for individuals seeking herbal remedies for wound healing.

Plant was collected in the month august and September near Nashik region of Maharashtra. Further its authentication done by Dr.V.B.Awale,,HOD of Botany, Dr.Pantangrao Kadam Mahavidyalaya,Sangli-416416, only fully grown healthy parts of plant were selected while damaged parts were rejected.

Procedure:

Morphological study of roots of *Glycyrrhiza glabra*: The organoleptic and morphological characters of the roots of *Glycyrrhiza glabra* were studied under dissecting microscope. **[Iyengar M A, *et.al*]**

Microscopical study of roots of *Glycyrrhiza glabra*

Powder characteristics of roots of *Glycyrrhiza glabra*: In present study the roots of *Glycyrrhiza glabra* were pulverized in to fine powder separately and the powder were investigated for their microscopic characteristics.

Procedure:

Glycyrrhiza glabra roots, were cooked separately with a small amount of a clarifying reagent, such as chloral hydrate solution. After the powder was cleaned, it was placed within a watch glass and stained using various staining reagents such as strong hydrochloric acid and phloroglucinol, iodine solution, Sudan red III, Ruthenium red, etc. For every slide, just one staining reagent should be used. A small amount of the powdered treatment was placed in diluted glycerin, and the slide was examined under a low-power microscope.

Processing of plant parts

Washing:

The roots of *Glycyrrhiza glabra* washed thoroughly with tap water to remove all dust & debris and seeds were separated from seed coat were shade dried.

Drying:

After washing the roots of *Glycyrrhiza glabra*, material was weighed and dried in shade for 5 – 10 days which was necessary to prevent the loss of chemical constituents. When all parts of crude selected drugs were dried it was again weighed and the dry weight of all parts obtained were noted.

Size reduction:

The roots of *Glycyrrhiza glabra* were reduced to a suitable size using willey grinder to the optimum grade, that gives most efficient extract.

Storage:

The powdered roots of *Glycyrrhiza glabra* were stored in a well closed container away from air and light in a cool shady place, till the extraction process.

Extraction procedure:

The coarse powder of roots of *Glycyrrhiza glabra* and the powdered parts were extracted by successive solvent extraction by using solvents viz Chloroform, Ethyl acetate, Ethanol, Methanol (50%), and water. Extractions were carried out by continuous hot percolation using Soxhlet apparatus & clavenger apparatus. After completion of extraction with one solvent, it was filtered and the marc left after chloroform was dried and extracted with ethanol and simultaneously with 50% methanol, ethyl acetate and water. The extract obtained from each extractions were filtered and the solvents were removed by distillation under reduced pressure. The extracts were concentrated in waterbath at 40^{o}C and dried in hot air oven at 40^{o}C.The dried extracts were powdered, packed and stored in desiccators and essential oil were collected by clavenger apparatus.

Solubility testing of the extract of roots of *Glycyrrhiza glabra* were carried out by using various solvents.

TLC PROFILE OF ROOTS OF *GLYCYRRHIA GLABRA:*

All the extract of roots of *Glycyrrhiza glabra* were subjected to thin layer chromatographic studies, to find out the probable number of compounds present in them. The adsorbent used for thin layer chromatography was silica gel G. The precoated TLC plates (Merk,Germany) were heated in an oven for 30 minutes at 110°C for activation. 5 ml of the test sample (1mg/ml in methanol) was

applied in the form of bands using capillary tubes. Mobile phases used for detection of phytoconstituents were given below in detail.

TLC Profile of *Glycyrrhiza glabra Linn*.

TLC analysis of the ethanol extract produced in the toluene mobile phase: TLC Profile of Glycyrrhiza glabra Linn. Ethyl acetate: Acetic Acid: 5.0: 4.2: 0.8 and detected under UV 254 and UV 366 nm. After derivatization with iodine.

UV analysis: Glycyrrhizin:

With the chemical C-B diluted in ethanol, there were noticeable peaks at 276 nm in a UV-Vis spectrophotometer.

FTIR Spectrum of the prepared formulations.

The FTIR spectra show that the following are the predominant peaks: 771.55, 1049.31, 1228.70, 1330.93, 1701.27, 2731.29, 2953.12, 3209.66, 3227.02, 3427.62cm-1. These peaks correspond to the probable functional groups.

Mass spectrum: Using uniform GC-MS parameters, the mass spectra of glycyrrhizin was captured on the Shimadzu GCMS-QP2010 Ultra for the GC-MS study of L-A. The NIST08 library was used to analyse the acquired spectra. The mass spectrum from the small mass (m/z) 50 and high mass (m/z) 503 is displayed in the GC-MS peak. Since electron ionisation (EI) of roughly 70 eV was used for GCMS analysis, only EI mass spectrometry can be used to interpret mass spectra.

HPLC Profile of Compounds: Glycyrrhizin:

Diamonsil C_{18} column; mobile phase: acetonitrile; gradient elution: 0~30 min: 32% B; 30~60 min: 75% B. Detection wavelength: 250 nm. Flow rate: 1.0 mL/min; column temperature: 30°C, injection volume: 20 μl. HPLC chromatograms

ACUTE DERMAL TOXICITY (OECD GUIDELINES 402)

Drug:

Essential oil & hydro alcoholic extract

Glycyrrhiza glabra L

Group: *Glycyrrhiza glabra L* root oil and hydro alcoholic extract

4.15.1. EXPERIMENTALANIMALS

Rats: Young adult nulliparous Wistar albino females, weighing between 95 and 105 g at the beginning of the experiment, were obtained from Manuthy, Thrissur District s College of Veterinary and Animal Science. The Institutional Animal Ethics Committee (IAEC) of CARE KERALAM Ltd. has evaluated and approved the current study protocol (CKL/TOX/IAEC/44-15). The animals were kept in typical laboratory settings, which included an air-conditioned room with a sufficient supply of fresh air provided by an IVC system (15 air changes per hour), a temperature range of 21.0 to 24.0 °C, and a relative humidity of 57% to 65%.

Every day, the relative humidity and temperature were noted. A single animal was kept in a typical polysulphonate cage (L 300 x B 170 x H 140 mm) with a top grill mesh made of stainless steel. The cage had compartments for holding pelleted food and water in a bottle that had a stainless steel sipper tube. Paddy husk that had been sterilised was supplied for bedding. The animals were kept in the lab for a minimum of five days after being acclimated, and every day they were checked for clinical symptoms. On the day of reception and the fifth day of acclimatization, a veterinarian examined each animal.

During the period of acclimatization and study, the animals were provided with unlimited food. Rodent feed from Amrut Lab was given. Throughout the period of acclimatization and study, water was freely available. The deep bore-well water, which was supplied in plastic water bottles with stainless steel sipper tubes, was exposed to ultraviolet light before being run through an activated charcoal filter in an Aqua guard water filter/purifier (produced by Eureka Forbes Ltd., Mumbai).

DERMALTOXICITY

The OECD guideline was followed in conducting the acute cutaneous toxicity test with the single highest test dose. Prior to the experiment, at least five days were spent selecting and acclimating Wistar Albino rats to the laboratory environment. All animals had their fur removed from the dorsal area of their trunks by clipping or shaving; caution was taken to prevent skin abrading, and only animals with unbroken skin were used in this investigation. Each animal s body surface area was cleansed of at least 10% before the control and test compounds were evenly placed across the removed region.

The creatures were split up into two groups. An essential oil and hydro alcoholic extract of the roots of *Glycyrrhiza glabra* served as the test item and a basic ointment foundation. Over the course of a 24-hour exposure

period, group I and group II applied 2000 mg/kg body weight dermally over an area covered with a porous gauze dressing and non-irritating tape, respectively. Throughout the course of the 14-day observation period, all of the animals were checked for clinical symptoms of toxicity and death every 30 to 40 minutes, 1 hour, 2 hours, 3 hours, and 4 hours after the dose.

Procedure:

A single administration of each doses of essential oil with hydro alcoholic extract 2.5, 5, and 10 gm/kg of body wt. in a final volume of 1ml was done in palm oil on skin of test animals in a group of 2, 3, 4 respectively.

The essential oil was prepared in palm kernel oil for various doses.

Control group animals applied only palm kernel oil.

Parameters:

The animals were observed daily for 14 days to detect possible behavioral changes including Salivation, appearance of the treated skin, respiration, state of the excrement, sleep, appearance of fur. Activity (locomotion), sensitivity of noise, reaction to pinch reactivity.

Table 1. Preparation of film-forming spray with different polymer types and concentrations

Ingredients	**Formulation(%w/w)**									
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**
PVPK30	1.0	2.0	3.0	4.0	5.0	-	-	-	-	-
HPMCE5	-	-	-	-	-	1.0	2.0	3.0	4.0	5.0
Purified water	49.5	49.0	48.5	48.0	47.5	49.5	49.0	48.5	48.0	47.5
Ethanol	49.5	49.0	48.5	48.0	47.5	49.5	49.0	48.5	48.0	47.5

Table 2: Making a film-forming spray using several plasticizers and essential oil and hydro alcoholic extract of liquorice

Ingredients	**Formulation(%w/w)**			**Function**
	L1	**L2**	**L3**	
PVP K30	3.0	-	3.0	Film forming agent

HPMC E5	-	2.0	-	Film forming agent
Propylene Glycol	1.0	1.0	-	Plasticizer
Polyethylene glycol 400	-	-	1.0	Plasticizer
Methyl salicylate	3.0	3.0	3.0	Active ingredient
Menthol	1.0	1.0	1.0	Active ingredient
Glycerrhiza glabra.	0.02	0.02	0.02	Active ingredient
Purified water	37.98	40.98	37.98	Solvent
Ethanol	54.0	52.0	54.0	Solvent

Preparation of film forming spray:

The spray was created with a simple solution procedure. The polymeric solution system was first created by dissolving polymers in a specific volume of water using a magnetic stirrer. A polymeric solution was added to each oil after it had been dissolved in ethanol. After that, plasticizer was added and diluted with water to reach the desired final weight. Topical film-forming sprays made from various raw medicines were produced after the excipients (plasticizers and polymers) in the formulation were screened. The formulations were supplemented with additional ingredients, including methyl salicylate as an excipient and menthol as a counterirritant, which were fully mixed in.

Evaluation of film forming spray formulations:

1. **Physical characteristics** After the prepared film-forming spray was left at room temperature (30±2 °C) for 0,7,14, and 28 days, the white spot on the surface, the film s thickness, and the clarity of the solution were all visually assessed.

2. **Evaporation time** Film-forming spray was used on bagasse paper that was suspended in a fume hood from a sensitive balance. The weight loss of the solvent liquid and bagasse paper is determined as a function of time as the solvent evaporates using an analytical balance.

3. **Volume per spray** For the spray formulations, the following quantitative tests were also run. The average weight per dosage is a crucial quantitative factor that needs to be assessed. A glass

beaker with ten sprays was filled with an analytical balance to determine the volume of each spray.

4. pH In a 30 mL glass beaker, around 20 mL of the film-forming spray solution was added. A pH metre (Seven Compact Mettler Toledo) was used to measure the pH. Each formulation s pH was measured three times, and the mean results were computed. pH values were collected for 0,7,14, and 28 days.

Table 3: Physical appearances of Liquorice film forming spray Formulation

Formulation	**Days**			
	0 day	**7 days**	**14days**	**28days**
L1	clear, thin film, smooth	clear, thin film, smooth	clear, thin film, smooth	clear, thin film, smooth
L2	clear, thin film, smooth	clear, thin film, smooth	clear, light yellow solution, thin film, smooth	clear, lightyellow solution, thin film, smooth
L3	clear, thin film, smooth, white spot	clear, thin film, smooth,white spot	clear, light yellow solution, thin film, smooth, white spot	clear, light yellow solution, thin film, smooth, white spot

Table 4: Evaluation of Liquorice oil film forming spray formulation

Formulation	**Evaluation**					
	Evaporation Time (Seccond)	**Volume Per Spray (Gm)**	**pH**			
			0 day	**7 days**	**14 days**	**28 days**
L1	27.78	0.11	6.40	6.38	6.40	6.40
L2	18.61	0.11	5.17	5.32	5.78	5.81

L3	14.**60**	0.11	5.08	5.**20**	5.45	5.68

PHARMACOLOGICAL ACTIVITIES:

1. Wound healing Activity Procedure: [Chidambara Murthy.*et al*.,2004], Villegas, L.F.*et al.,1997]*

Plants that hasten the healing process of wounds, such as *Glycyrrhiza glabra* was chosen for this study. A wound is the result of physical, chemical, electrical, or microbiological shocks to live tissue that break the cellular, anatomical, and functional continuity of the tissue. The dynamic process of mending a wound involves the regeneration or repair of damaged tissue. The typical wound-healing response is a coordinated series of processes that start with the damage.

When the platelets come into contact with exposed collagen, they aggregate and release clotting factors, which causes a fibrin clot to form at the site of injury. This initiates the healing cascade. The fibrin clot acts as a temporary matrix and prepares the body for the healing processes that follow. At the site of injury, inflammatory cells also accompany platelets, delivering vital messages called cytokines or growth factors. The connective tissue called fibroblast is in charge of depositing the collagen required to repair tissue damage.

Collagen gives tissues their strength, integrity, and structure. Collagen is required to repair defects and restore anatomic form and function when tissues are damaged after an injury. In the current study, formulations were created to hasten the healing of wounds. *Glycyrrhiza glabra* have antibacterial, antioxidant, anti-inflammatory, and wound-healing properties, as well as an increase in collagen synthesis and tensile strength, according to a literature review. In the current investigation, wound healing activity in wistar rats was used to evaluate the feasibility of wound film forming sprays, which were produced for simple application, to povidone iodine ointment.

2. Evaluation of Wound Healing Activity:

The Committee of Purpose of Control and Supervision of Experiment on Animals (CPCSEA) Bharat criteria were followed by the institutional animal ethical committee when approving the experimental procedure. For the investigation, albino rats (Wistar strain, 180–220 g) were employed. Water and pelleted food were available to all animals at all times. The temperature remained constant at 23±1°C.

Animals were inflicted with semi-aseptic wounds while under light ether anesthesia. Five

groups (n=6) of animals were assigned to the animals. As group I received no treatment, this was regarded as the control. Animals in Group II were treated with Povidone iodine ointment as usual. In all excision models, test formulations I and II, III, and IV were administered to animals in groups III, IV, V, and VI, respectively. Throughout this trial, the animals received no more systemic or topical treatments.

Excision Wound Healing Procedure:

Six rats were given diethyl ether inhalation anesthesia, and their backs were shaved after being sterilized with 70% ethanol. A back imprint was taken, placed 5 cm from the ear and 1 cm from the spinal column. The entire thickness of the skin, or roughly 500 mm2, was removed from the impressed wound area.The wound was covered with medication twice a day. The first day, designated as day 1, was used to produce the wound. On each subsequent day, the wound contraction was measured on the corresponding days (1, 3, 8, 13, 18, 21), and the impression was recorded on paper.

Drawing the raw wound area on a clear sheet of paper every other day starting on the day the incision was made and continuing until the wound healed totally, whichever occurred first, allowed researchers to study contraction, which primarily helps in wound closure. measurement of the contraction of the wound. The trace of the wound was copied to a one mm square piece of graph paper in order to calculate the wound area. The wound contraction was calculated using the equation as a percentage of the initial wound size, which was taken to be 100% for each animal in the group.

Table 5: Group wise distribution of animals for wound healing activity [Excision wound model]

Group	1st day	3rd day	8th day	13th day	18th day	21st day	% Wound contraction
Control	21.6± **0.11**	21± **0.2**	18.7± **0.14**	14.3± 0**.17**	8.3± **0.28**	6.4± **0.11**	70.37± **0.23**
Standard (Povidone-Iodine)	**20.9**± **0.05**	**19.3**± **0.05**	**13.2**± **0.26**	**11.5**± 0.23	**7.2**± 0.34	**3.2**± 0.03	84.68± 0.05
Test (Liquorice)	21.3± 0.02	19.7± 0.04	18.4± 0.05	13.3± 0.23	10.6± 0.6	7.6± 0.06	64.31± 0.03

Each group s values consist of the mean ± SEM of six animals (n = 6). The proportion of wound contraction is shown by the number in parenthesis. When compared to group I (control), all of them are significant at $P<0.05$, which means they are not significant.

The number of days needed for the Escher—dead tissue observed in a full-thickness wound—to fall off and leave no raw wound area behind was used to track the epithelialization process.

RESULTS AND DISCUSSION

PHARMACOGNOSTIC EVALUATION:

Morphology of Liquorice root:

- Odour : Faint and Characteristics
- Colour: Yellowish brown
- Taste: sweet.
- Shape: Cylindrical
- Size: 20-40 cm in length and 2cm in diameter.

Microscopy of Liquorice root:

- Periderm (Phellem)(Cork): Tabular cells fitted with reddish brown content.
- Phellogen: Below cork parenchymatous cells with cellulose and calcium oxalates.
- Secondary Phloem: Bundles of fibres surrounded by a parenchymatous sheath containing calcium oxalates.
- Medullary rays: multistearate and paranchymatous . Rays are narrow in xylem and wise in phloem.
- Secondary xylem-Xylem consist of vessel, fibres and lignified wood parenchyma. Starch is present in wood parenchyma.
- Pith: consist of large parenchyma with intercellular spaces and few starch grains Pith is absent in root.

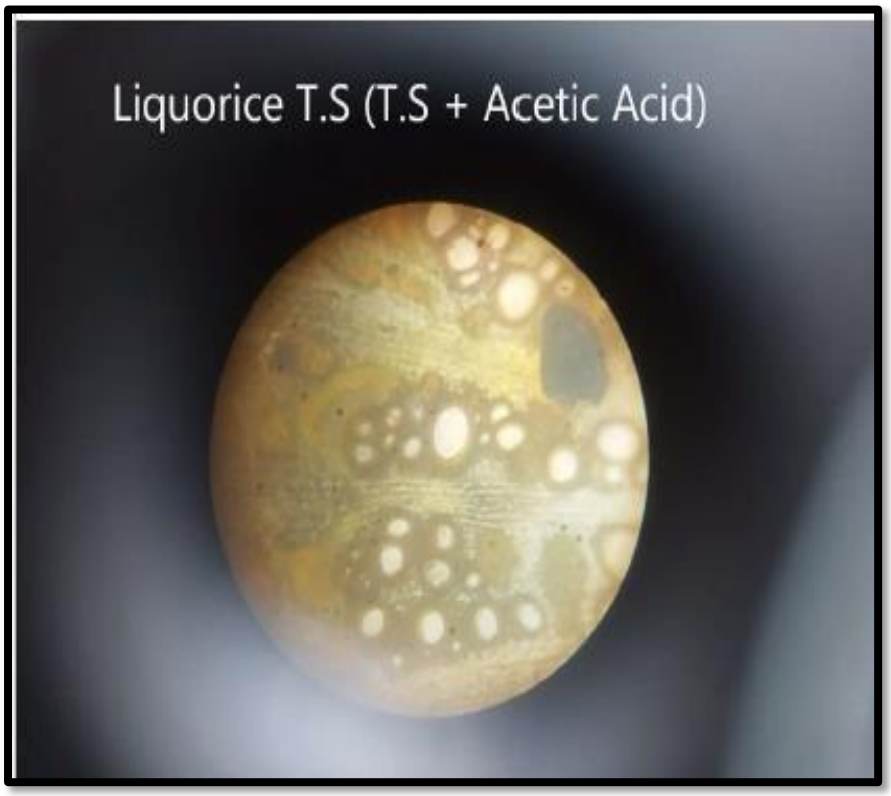

Figure 1 :T.S.of Liquorice root with Acetic acid

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Figure 2 :TS of Liquorice root with Dil HCl

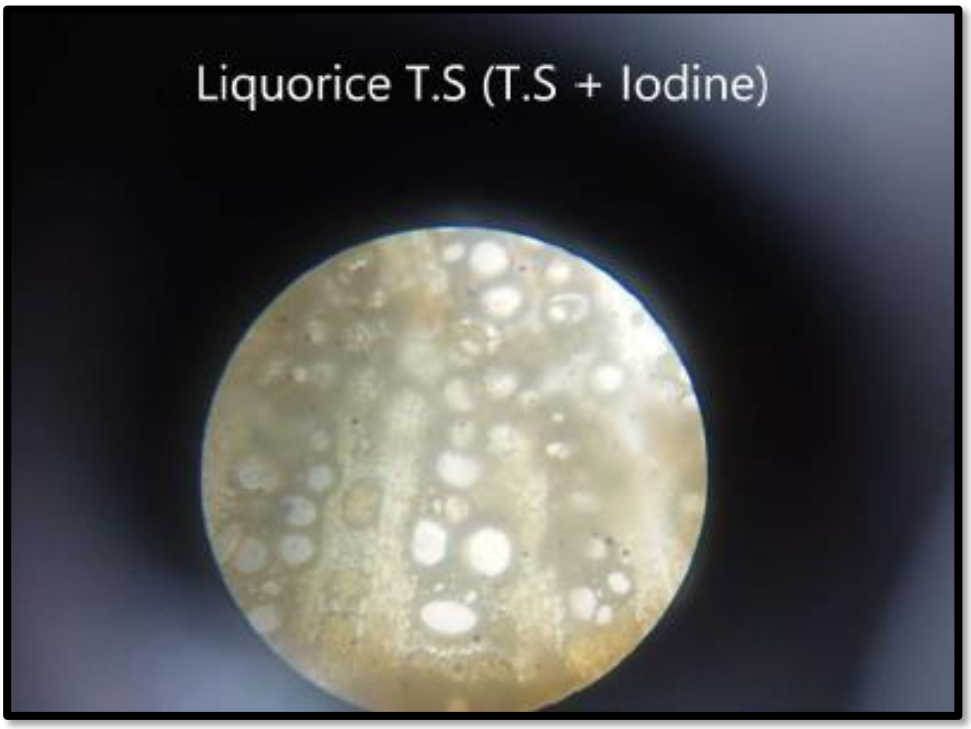

Figure 3:T.S. of Liquorice root with Iodine solution

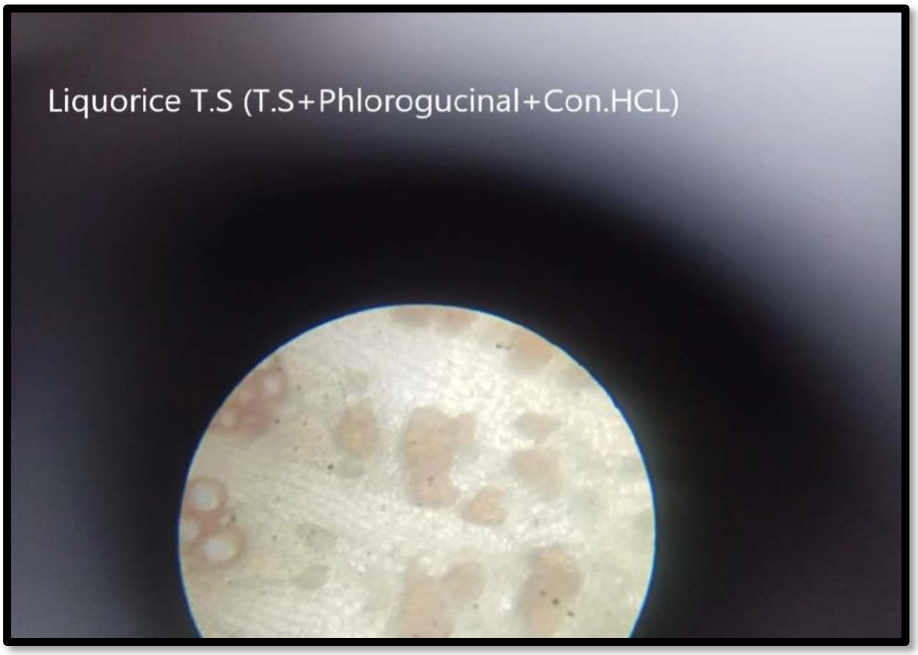

Figure 4:T.S. of Liquorice root with Phloroglucinol +Conc HCl (1:1)

5.5. PHYTOCHEMICAL EVALUATION

Table 4.1: Solubility profile of roots of G*lycyrrhiza glabra*

S.No	Reagents	Roots of *Glycyrrhiza glabra*
1.	Distilled Water	-
2.	Acetone	++
3.	Methanol	+
4.	Benzene	+
5.	Chloroform	++
6.	Ethanol	+
7.	Acetic acid	++
8.	Petroleum ether	+
9.	DMSO	-
10.	Ethyl acetate	++

Table 7: Preliminary phytochemical screening for hydro-methanolic root extract of *Glycyrrhiza glabra* Linn.

S.no	Phytoconstituents	Testperformed	Result
1.	Carbohydrates	Molisch stest	**(-)**
2.	Proteins	Copper sulphate test	**(-)**
3.	Flavonoids	Lead acetate test, NaOH solution test	**(+)**
4.	Alkaloids	Dragendroff stest	**(+)**
5.	Steroids	Lieberman stest	**(+)**
6.	Terpenoids	Salkowski stest	**(+)**
7.	Saponins	Frothtest	**(+)**
	Tannins	Ferric chloride test	**(+)**
9.	Phlobatannins	HCLtest	**(-)**
10.	Anthraquinones	Benzenetest	**(-)**
11.	Glycosides	Keller-Killanitest	**(+)**
12.	PhenolicCompounds	Ferricsulphatetest	(-)

Table 8: Compiled results for total ash, acid insoluble ash, water soluble extractives and alcohol soluble extractive values as compared to standard values for root extract of *Glycyrrhiza glabra* Linn.

S. No.	TESTS	OBSERVATIONS(%)	STANDARD % (API)
1	Total ash	3.75	Notmorethan10
2	Acid-insoluble ash	1.93	Notmorethan2.5
3	Water soluble extractives	3.51	Notlessthan20

4	Alcohol soluble extractives	2.18	Notlessthan10

Table 9: Loss on drying observations at different time intervals root extract of *Glycyrrhiza glabra* Linn.

Sr.no	Time (h)	Weight (g)
1.	0	64.75
2.	1	64.38
3.	2	64.21
4.	3	64.15
5.	4	64.08
6.	5	64.10
7.	6	64.10

TLC Profile of Glycyrrhiza glabra Linn. :

TLC analysis of the ethanol extract produced in the toluene mobile phase: Acetic acid: Ethyl acetate: 5.0: 4.2: 0.8 was observed under UV 254 nm, revealing 5 spots at Rf values of 0.22, 0.44, 0.69, and 0.70 (green colour). Under UV 366 nm, 12 spots were revealed at Rf values of 0.06, 0.18, 0.28, 0.35, 0.43, 0.50, 0.58, 0.63, 0.67, 0.72, and 0.79 (blue), and 8 spots were revealed at Rf values of 0.22, 0.31, 0.46, 0.53, 0.62, 0.68, 0.73, and 0.86 after derivatization with iodine. It has been demonstrated that each of these phytochemicals has a wide range off biological effects, including potent antioxidant and anti-inflammatory and wound-healing capabilities.

CHARACTERIZATION OF ACTIVE CONSTITUENTS BY USING VARIOUS SPECTRAL ANALYSIS.

In the present investigation UV , HPLC, IR, Mass methods were employed to characterize the different extracts of root. The details of the investigation are given below

5.7.1. Glycyrrhizin from *Glycyrrhiza glabra*:

Results of UV-VIS spectroscopy

Absorbance Maxima (λ_{max}): 254 nm

When the compound L-A was diluted in ethanol, a UV-Vis spectrophotometer exhibited distinct peaks at 254 nm.

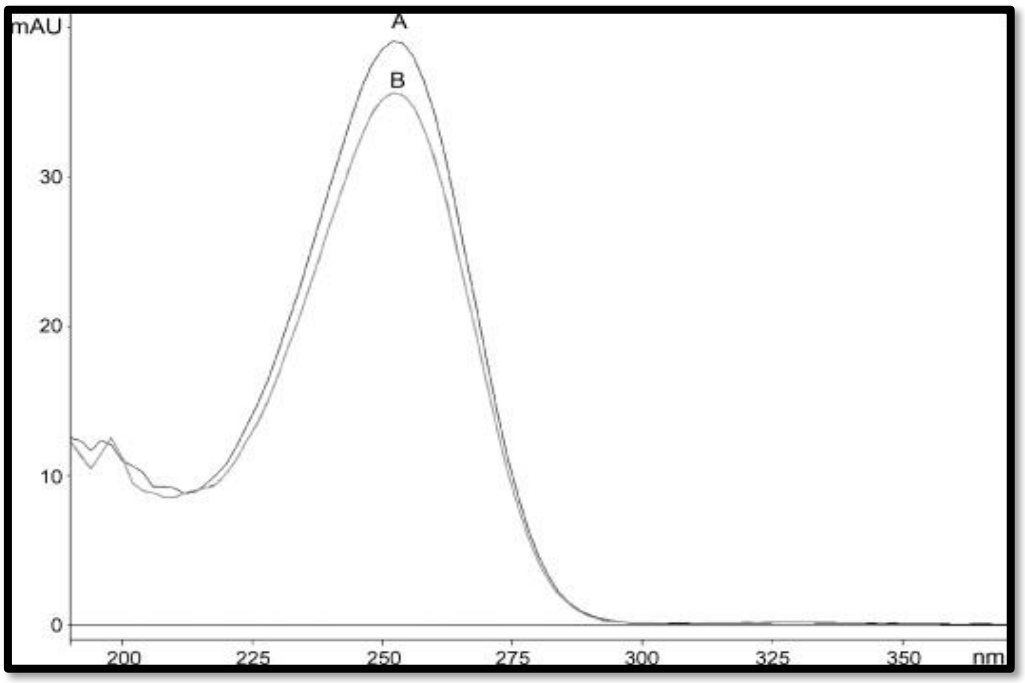

Figure 5:UV-VIS Profile of L-A

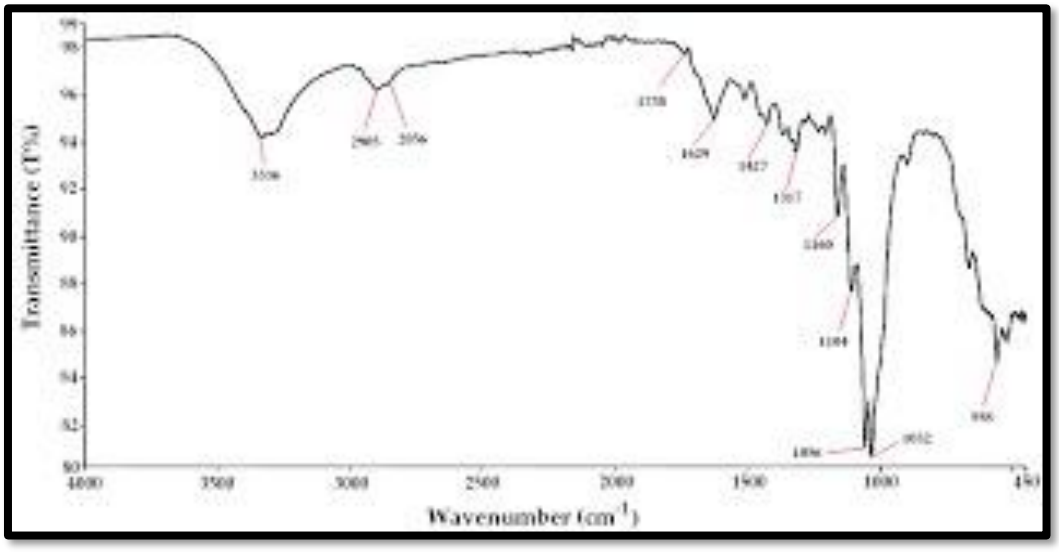

Figure 6: FTIR spectrum of compound-I.

The FTIR spectra show that the following are the predominant peaks: 771.55 1049.31 1228.70.1330.93 1701.27 2731.29 2953.12 3209.66.3227.02 3427.62cm-1. The IR frequencies and the most likely functional groups are related.

Table 10: Results of FTIR

Frequency (cm^{-1})	Functional Group
771	C=C-H Bend
1049	C-O Stretch
1701	C=0 Stretch
2753	CH2, CH3 Stretch
3209	C-H stretch attached to C=C
3427	C-OH

The GC-MS analysis of L-A was performed utilising the Shimadzu GCMS-QP2010 Ultra with consistent GC-MS settings. The NIST08 library was used to analyse the acquired spectra.

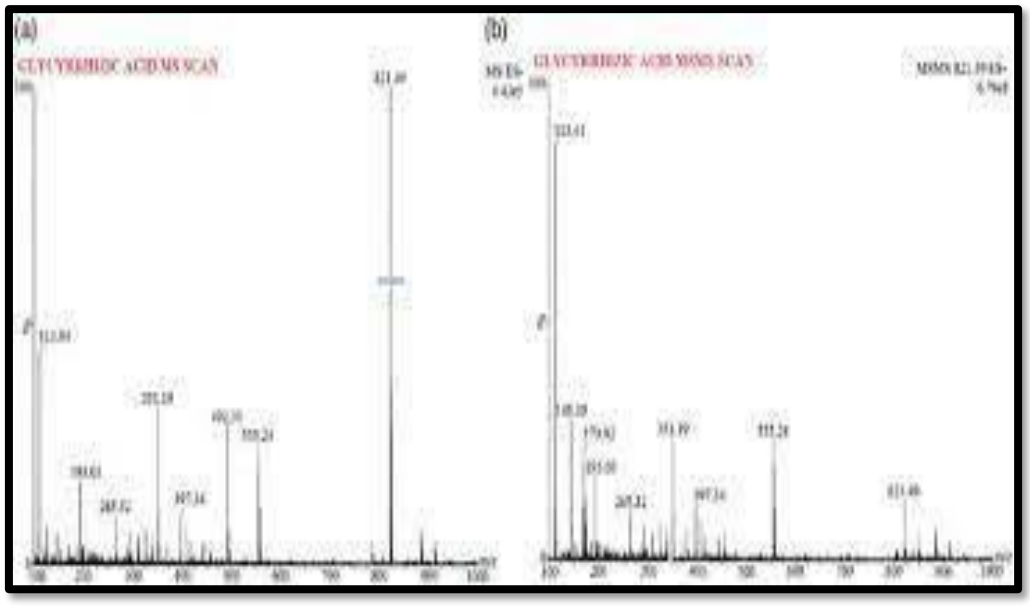

Figure 7:Fragmentation Pattern: Mass

The GC-MS peak shows the mass spectrum from the small mass (m/z) 50 and high mass (m/z) 503.

Table 11: GCMS results

Sr. No.	Fragments (m/z)
1	821.3948 999 822.3992 426 823.4048 77

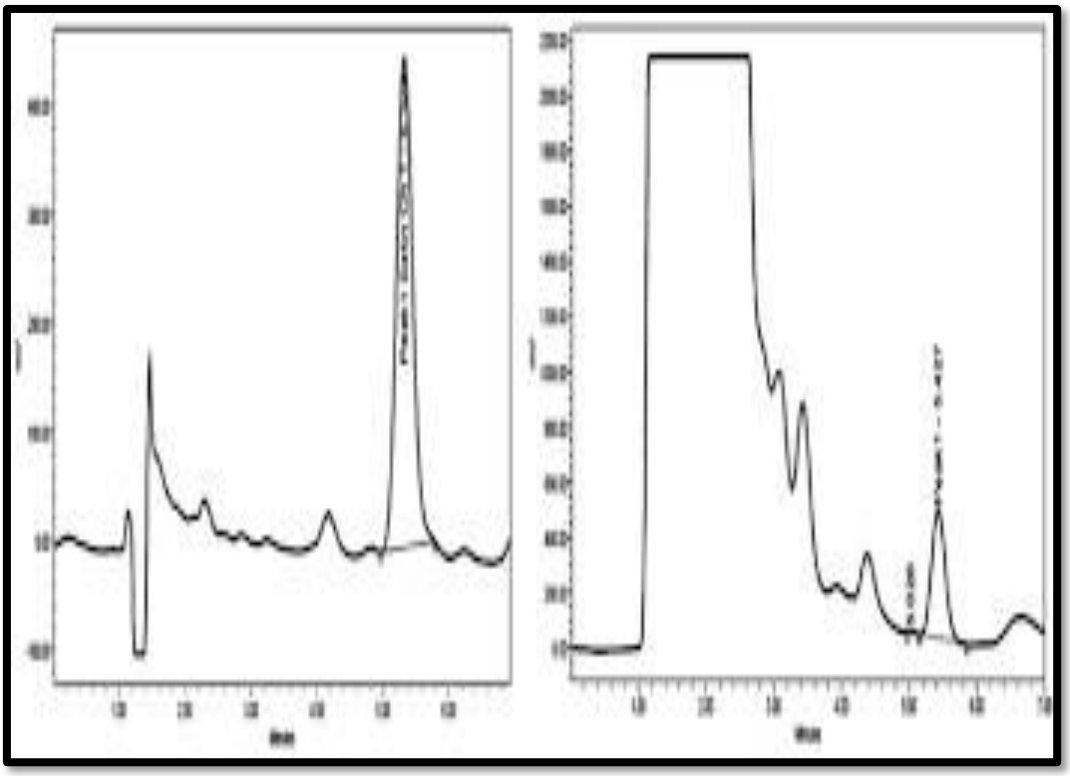

Figure 8: HPLC Profile of Compound L-A

Diamonsil C_{18} column; mobile phase: acetonitrile; gradient elution: 0~30 min: 32% B; 30~60 min: 75% B. Detection wavelength: 250 nm. Flow rate: 1.0 mL/min; column temperature: 30°C, injection volume: 20 μL. HPLC chromatograms are shown in **Figure** Fragmentation Pattern

Preparation of film forming spray using various types of plasticizer using Liquorice essential oil.

Table 12: Preparation of film forming spray using various types of plasticizer using Liquorice essential oil and hydro alcoholic extract.

Ingredients	**Formulation (%w/w)**			**Function**
	L1	**L2**	**L3**	
PVP K30	3.0.	--	3.0	Film forming agent
HPMC E5	--	2.0	--	Film forming .agent
Propylene G lycol	1.0	1.0	--	Plasticizer
Polyethylene gl ycol400	--	--	1.0	Plasticizer
Methyl Sali cylate	3.0	3.0	3.0	Active ingredient
Menthol	1.0	1.0	1.0	Active ingredient
Glycyrrhiza glabra oil & hydro alcoholic extract	0.02.	0.02	0.02	Active ingredient
Purified Water	37.98	40.98	37.98	Solvent
Ethanol	54.0	52.0	54.0	Solvent

Preparation of film forming spray: The spray was made using a straightforward solution technique. Initially, a magnetic stirrer was used to dissolve polymers in a certain amount of water and create the polymeric solution system. A polymeric solution was added to each oil after it had been dissolved in ethanol. After that, plasticizer was added and diluted with water to reach the desired final weight. The topical film-forming sprays from several crude pharmaceuticals were generated after the excipients (plasticizers and polymers) were screened. To finish the formulations, other ingredients were added and carefully combined, including methyl salicylate and menthol as counter irritants, both of which dissolved in

ethanol.

Each film-forming spray composition possessed unique physical properties. Each film-forming spray solution preparation was documented in a table and stored in a hermetically sealed container equipped with a spray pump, as depicted in Figure. The assessment of the film-forming spray compositions was shown in a table.

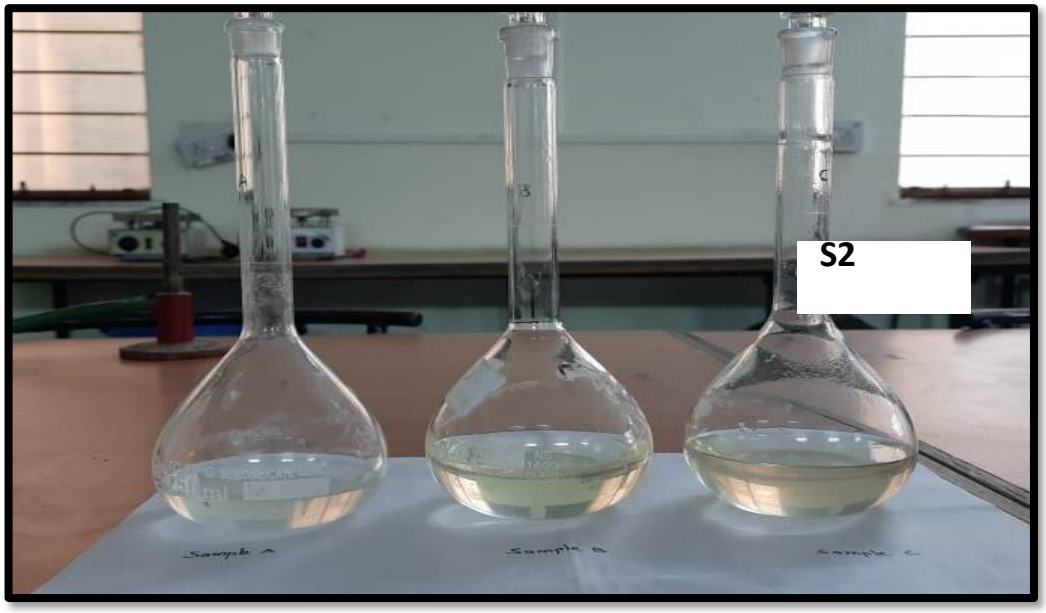

Figure 9: Formulation of different film forming spray.

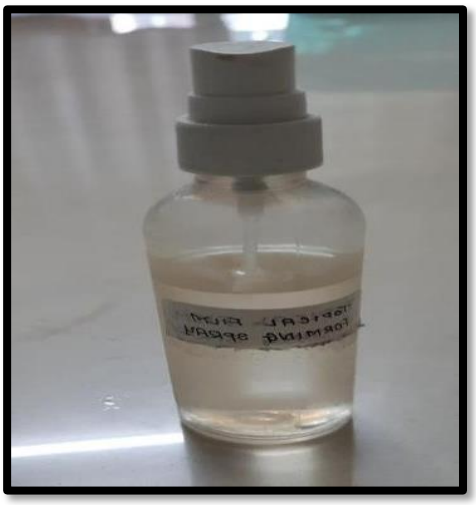

Figure 10: Film Forming Spray with Container

EVALUATIONOF FILMFORMING SPRAY FORMULATIONS

1. Physical characteristics: A visual assessment was conducted to determine the thickness of the film, the clarity of the solution, and the white spot on the surface of the prepared film-forming spray after it was left at room temperature (30±2 °C) for 0, 7, 14, and 28 days.

2. Evaporation time: Bagasse paper was suspended in a fume hood from a sensitive balance and treated with film-forming spray. Using an analytical balance, the weight loss of the solvent liquid and bagasse paper is calculated as a function of time as the solvent evaporates.

3. Volume per spray: We also conducted the following quantitative tests for the spray formulations. One important quantity that must be evaluated is the average weight per dosage. To find the volume of each spray, an analytical balance was placed inside a glass beaker containing ten sprays.

4 pH- About 20 ml of the film-forming spray solution were added to a glass beaker with a capacity of 30 ml. The pH was measured using a Seven Compact Mettler Toledo pH metre. The pH of each formulation was measured three times, and the average was calculated. pH readings were taken every 0, 7, 14, and 28 days.

Table 13: Liquorice film-forming spray formulations outward appearance

Formulation	**Days**			
	0 day	**7 days**	**14 days**	**28days**
L1	Clear, thinfilm, smooth	Clear, thinfilm, smooth	Clear, thinfilm, smooth	Clear, thinfilm, smooth

L2	Clear, thinfilm, smooth	Clear, thinfilm, smooth	Clear , lightyellow solution,thin film, smooth	Clear , light yellowsolution,thin film,smooth
L3	Clear, thinfilm, smooth white spot	Clear, thinfilm, smooth white spot	Clear, light yellowsolution, thin film, smooth, white spot	Clear, lightyellow solution,thin film , smooth, whitespot

Table 14: Evaluation of Liquorice oil & hydro alcoholic film forming spray formulation

Formulation	**Evaluation**					
	Evaporation Time (Second)	**Volume Per Spray (g m)**	**pH**			
			0day	**7days**	**14 days**	**28 days**
L1	27.78	0.11	6.40	6.38	6.40	6.40
L2	18.61	0.11	5.17	5.32	5.78	5.81
L3	14.**60**	0.11	5.08	5.**20**	5.45	5.68

Evaluation of Wound Healing Activity:

The Committee of Purpose of Control and Supervision of Experiment on Animals (CPCSEA) Bharat criteria were followed by the institutional animal ethical committee when approving the experimental procedure. Albino rats (180–220 g; Wistar strain) were the animals used in the research. Water and pelleted food were available to all animals at all times. The temperature remained constant at 23±1°C.

Animals were inflicted with semi-aseptic wounds while under light ether anaesthesia. Five groups (n = 6) were assigned to the animals.As group I received no treatment, this was regarded as the control. Animals in Group II were treated with Povidone iodine ointment as usual. In all excision models, test formulations I and II, III, and IV were administered to animals in groups

III, IV, V, and VI, respectively. Throughout this trial, the animals received no more systemic or topical treatments.

Excision Wound Healing Procedure:

Six rats were given diethyl ether inhalation anesthesia, and their backs were shaved after being sterilized with 70% ethanol. A mark was made in the back, 5 cm from the ear and 1 cm from the spinal column. The entire thickness of the skin, or roughly 500 mm2, was removed from the impressed wound area. The wound was covered with medication twice a day. The first day, designated as day 1, was used to produce the wound. On each subsequent day, the wound contraction was measured on the corresponding days (1, 3, 8, 13, 18, 21), and the impression was recorded on paper.

A clear piece of paper was used to trace the raw wound region every other day starting on the day the incision was made and continuing until the 21st postoperative day, or, if that happened sooner, until the wound closed completely, in order to study contraction, which largely helps in wound closure. measurement of the contraction of the wound The trace of the wound was transferred to a graph paper measuring one mm square in order to determine the extent of the wound. The wound contraction was calculated using the equation as a percentage of the initial wound size, which was taken to be 100% for each animal in the group.

Using the formula, the wound s contraction was determined:

$$\text{Wound percentage} = \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

The number of days needed for the Escher—dead tissue observed in a full-thickness wound—to fall off and leave no raw wound area behind was used to track the epithelialization process.

Group	1st day	3rd day	8th day	13th day	18th day	21st day	% Woundcontraction
Control	21.6±0.1155	21±0.2	18.7±0.14	14.3±0.170	8.3±0.28	6.4±0.11	70.37±0.23
Standard (Povidone-Iodine)	20.9±0.05	19.3±0.05	13.2±0.26	11.5±0.23	7.2±0.34	3.2±0.03	84.68±0.05
Test (Liquorice)	21.3±0.02	19.7±0.04	18.4±0.05	13.3±0.23	10.6±0.6	7.6±0.06	64.31±0.03

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