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Formulation and evaluation of in-vivo antibacterial activity of herbal gel containing leaf extract of *Oroxylum indicum* (L.) Kurz

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ABSTRACT: Herbal medicine has a remarkable role in treatment of diseases in human as well as in animals and become globally important in both medicinal and economical field. Herbal medications are considered safer than allopathic medicines as herbal medications associated with minimal side effects. Preparation of extract and their formulations for better absorption and penetration of the active moiety into the systemic circulation can increase its survival. The present research has been undertaken to formulate and characterize an antibacterial gel using the hydroalcoholic leaf extract of *Oroxylum indicum.* The fresh leaves were collected and extracted by Soxhlation using water:ethanol (70:30) as solvent. The gel formulation was designed by using Carbopol 934 polymer, sodium carbonate and required amount of distilled water. The skin pH was maintained by drop wise addition of Tri-ethanolamine. The physicochemical parameters of the formulation were determined. The interaction study between extract and excipients was done by FTIR spectroscopy.
KEYWORDS: *Oroxylum indicum*, extraction, formulation, herbal gel, antibacterial efficacy.

I. INTRODUCTION

Medicinal plants as a source for therapeutic aids has attained a remarkable role in human as well as in animals not only in diseased condition but also as potential material for maintaining proper health[1]. Over the past several years, great advances have been made on development of a herbal gel from the plant activities and extracts. Topical gel preparations are considered for skin application for local action or percutaneous penetration of medicament. Topical gels as wound healing treatment are popular because they are cost-effective, easy to use and comfortable for the patients[2]. *Oroxylum indicum (*Bignoniaceae) is a species of flowering traditional medicinal plant which is commonly known as Bhat-ghila or Takuna in Assam. This plant is native to the Indian subcontinent, the Himalayan foothills with a part extending to Bhutan and southern China, Indochina and the Malaysia regions. It is visible in the forest biome of Manas National Park in Assam.

Bark is used in internal abscess, hypertension and ulceration in mouth of children and babies, seeds used in eczema. Decoction of leaves use internally to increase appetite. The present study was conducted to formulate the herbal *Oroxylum indicum* topical gel formulation against skin infections caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*. In this study an antibacterial gel formulation will be developed with the hydro-alcoholic extract of *Oroxylum indicum* and its *in-vivo* efficacy will be evaluated in rat models.

II. MATERIALS AND METHODS

Plant Materials and Preparation of Extract

Oroxylum indicum (L.)Kurz (Bignoniaceae) were collected from the local areas of Tengakhat, Dibrugarh, Assam and was authenticated at Botanical Survey of India, Eastern Regional Centre, Shillong-793003 and a voucher specimen was deposited, No.BSI/ERC/Tech//Identification/2017/707.

The leaves were washed thoroughly. The foreign, earthy matter and residual materials were removed carefully from the leaves and cleaned, dried in the shade. It was then powdered by using a mechanical grinder and stored in air tight containers free from moisture for further use. The powdered crude drugs were placed inside a thimble made from thick filter paper, which was loaded into the main chamber of the Soxhlet extractor and the temperature maintained at 25°C. The Soxhlet extractor was placed onto a flask containing the hydroalcoholic (water:ethanol, 70:30) solvent until it get exhausted. The solvent was recovered using the rotary evaporator and the remaining aqueous extract was dried using freeze dryer at -80°C and pressure maintained at 0.05 Mbar. It was stored in air-tight containers at 4-8°C until use.

Pre-Formulation Study

Pre-formulation study is a stage of formulation development in which the physicochemical evaluation of the drug substances and the plant extract alone as well as when it is combined with the excipients are investigated. It is the first step in development of dosage form as it may provide rationale for formulation design[17].This study mainly aimed at gathering of information useful to the formulator in developing acceptable, stable and efficacious dosage form. It includes the various analytical techniques such as Fourier Infrared Spectroscopy (FTIR), melting point and physical properties.

The FTIR spectra of the sample of plant extract was recorded by ATR (Attenuated total reflectance) technique in a FTIR spectrometer, over a range of 4000cm^{-1} to 400cm^{-1}. The spectrum was obtained by keeping the sample over the lens of the ATR probe. The melting point and physical properties were determined by capillary method and morphological studies respectively.

Extract Polymer Compatibility Studies

The interaction studies were carried out to ascertain any kind of chemical interaction of extract with the excipients used in the preparation of gel formulations. FTIR spectra were obtained by using a FTIR spectrophotometer at a resolution of 4cm^{-1} from 4000 cm^{-1} to 400 cm^{-1}.

Preparation of *Oroxylum indicum* Leaf Extracts Herbal Gels

The herbal gel formulation of *Oroxylum indicum* leaves extract was prepared with polymer Carbopol 934. Carbopol 934 was dispersed in distilled water (50 ml) with continuous stirring. 5 ml of distilled water was taken and required quantity was dissolved by adding Propylene glycol (5% w/w, as humectant). After that sodium carbonate (0.25% w/w, as preservative), was added and mixed properly. Further required quantity of *Oroxylum indicum* leaves extract was mixed uniformly in mortar and pestle to the above mixture and volume made up to 100 ml by adding remaining distilled water. Finally full mixed ingredients were mixed properly to the Carbopol 934 gel with continuous stirring and Tri-ethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel of required consistency. The prepared hydrogel were packed in wide mouth jar covered with screw capped plastic lid and were kept in dark and cool place. Samples were allowed to equilibrate for at least 24 h at room temperature prior to performing rheological measurement [9].

Characterization of Prepared Herbal Hydrogels

1. **Visual Examination**

The prepared herbal hydrogels were visually inspected for their color, homogeneity, grittiness and syneresis.

2. **pH Determination**

The pH of the gel formulations was determined using digital pH meter, which was calibrated before each use. Each measurement was carried out in triplicate and average pH was calculated.

3. **Determination of Spreadability**

Spreadability was determined by apparatus which was suitably modified in the laboratory for the study. It consists of a wooden block provided by a pulley at one end. Two sets of glass slides of standard dimensions were taken. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slide. 100gm weight was placed on the upper slides so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides were scraped off. The two slides in position were fixed to a stand without slightest disturbances in such a way that only the upper slide to slip off freely by the force of weight tied to it. A 20gm weight was tied to the upper slide to travel the distance of 7.5cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated by three times and the mean time was taken for calculation [10].

Spreadability was calculated by using the formula:

$$S = m \times \frac{l}{t}$$

Where,

S – Spreadabilty
M – Weight tied to the upper slide
l – Length of the glass
t – Time taken in seconds

4. Determination of Extrudability

Extrudability is the force required to exude material out of tube; determining the consistency of preparation. A closed collapsible tube containing about 20gm of gel was pressed firmly at the crimped end and a clamp was applied to prevent any rollback. The cap was removed and the gel was extruded until the pressure was dissipated [11].

5. Determination of Viscosity

Viscosity was determined using Rheometer (Brookfield R/S,USA) with spindle # C 50-1 having a speed of 50 rpm. All measurements were done in triplicate and the average reading is recorded at room temperature [9].

6. Stability Studies

O.indicum hydrogels were subjected to stability studies at refrigerator (4°C) and ambient room temperature (25°C) over a period of three months. After storage for three months, hydrogels are observed for any change in color, odor, pH, drug content, rheological properties and phase separation [13].

In-Vivo Studies on Antibacterial Activity

The pharmaceutical potential of *Oroxylum indicum* herbal gel as a novel local treatment for antimicrobial effect was investigated in a murine surgical site infection model.

Experimental Animals

Wister Albino rats of 150-200 gm; 4-6 weeks old (male and female) were used for the in-vivo experiments. All animals were purchased from Saha Enterprise, Kolkata, India and used for experimental study. All the protocols were approved by the Instutional Animal Ethics Committee (IAEC), Dibrugarh University, Assam and conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals); vide approval number 1576/GO/ERe/S/11/CPCSEA, dated: 30/3/2015.The animals were housed in large specious hygienic polypropylene cages and provided standard pellet diet and water and maintained at room temperature of 20-25 ± 2ºC, 30-35% of relative humidity with 12 hours light/dark cycle. Animals were kept for 1 week to acclimatize to laboratory conditions before starting the experiment, they were allowed to free access for water and standard rat feed.

At the end of experimental period the surviving animals were rehabilitated. They were provided normal feed, water and maintained for further use. All animal carcasses were packed in polythene bags and buried deep in the ground covered with lime and disinfectants.

Experimental Design:

A total number of eighteen Wister Albino Rats were divided into two groups of nine animals. These animals were again divided into six groups, each group containing three animals. Prior to the study, animals were fasted by depriving them of food overnight, allowing free access of water. To induce infection with the desired bacterial species, a cut of 1 cm length and depth of 2 mm (approx.) was given on the dorsal side of the animal, after shaving the hair. A piece of absorbable suture was dipped in the solution of the bacterial colony (*S.aureus* and *S. epidermidis*). This suture placed inside the cut was secured with stitches on the skin. The animals were grouped as Group I: control group (*S. aureus*), Group II: bacterial (*S. aureus*) +*O. indicum*, Group III: bacterial (*S. aureus*)+ standard drug, Group IV: control group(*S. epidermidis*), Group V: bacterial (*S. epidermidis*)+*O. indicum*, Group VI: bacterial (*S. epidermidis*) + standard drug. The animals were observed visually and by measuring the cut of 1 cm in every day by a scale for three weeks to check the efficacy of the plant formulation against skin disease.

Histological Study:

After completion of three weeks, to confirm the findings, bioburden study was performed by taking 1 cm^2 of animal tissue from the test animal. This was done by comparing the thickness of microbial bioburden between the treated and untreated tissues. Firstly the animal tissue was homogenate with a homogenizer and after continuous homogenization the supernatant was collected to count the bacteria by taking the supernatant into 10 fold dilution.

The standard plate count method was used for determining bacterial numbers. It consists of diluting the animal tissue with sterile saline or phosphate buffer diluent until the bacteria are dilute enough to count accurately that is, the final plates should have between 30 and 300 colonies. Fewer than 30 colonies are not acceptable for statistical reasons (too few may not be representative of the sample), and more than 300 colonies on a plate are likely to produce colonies too close to each other to be distinguished as distinct colony-forming units (CFUs). The assumption is that each viable bacterial cell is separate from all others and will develop into a single discrete colony (CFU). Thus, the number of colonies should give the number of bacteria that can grow under the incubation conditions employed. Using aseptic technique, a wide series of dilutions (e.g., 10^{-4} to 10^{-10}) is normally plated because the exact number of bacteria is usually unknown. Greater accuracy is achieved by

plating duplicates or triplicates of each dilution. The number of bacteria (CFU) per milliliter or gram of sample is calculated by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquefied agar [14].

$$\text{No. of bacteria/ml} = \frac{\text{numberofcolonies (CFUs)}}{\text{dilution} \times \text{amountplated}}$$

Statistical Analysis

The data obtained from the experiment were expressed as mean ± SEM. The significance of the difference between the means of test and control studies was established by student's t-test. P value less than 0.01, 0.05 were considered significant.

III. RESULTS AND DISCUSSION

Pre-Formulation Study

The FTIR of the *Oroxylum indicum* extract is shown in the figure 1. From the interpretation it was confirmed that the compound contain the hydroxyl O-H group at 3219 cm^{-1} and amine NH_2^+group at 2344 cm^{-1}. The presence of O-pyrone rings at 1282 cm^{-1}shows the presence of flavone, a flavonoid.

The melting point of the hydroalcoholic extract of the leaves of *Oroxylum indicum* was found to be 257°C.The physical properties of the leaves of *Oroxylum indicum* were observed and are as mentioned below in table1.

Table 1: Physical properties of *Oroxylumindicum* leaves

Sl no.	Characteristics	Leaves	
1	Color	Fresh	Dark green
		Dried powder	Dark green
2	Size	90-180cm	
3	Odor	Characteristic	
4	Taste	Bitter	
5	Surface	Smooth and hairless	

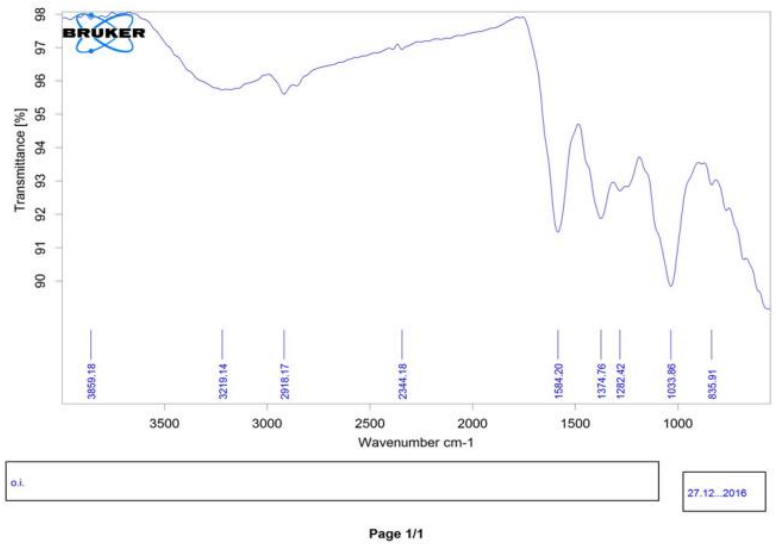

Figure 1: FTIR spectra of *O. indicum* leaf extract

Extract Polymer Compatibility Studies

FTIR analysis of *O.indicum* extract, the polymer (C934), physical mixture of extract with the polymer and the formulation was carried out to investigate the compatibility between the drug and the excipients. From the interpretation it was observed that there was no visible physical or chemical interaction between the drug and the excipients as there was no any considerable change in the peaks of drug was observed when mixed with the excipients. The IR spectra is shown in figure 2.

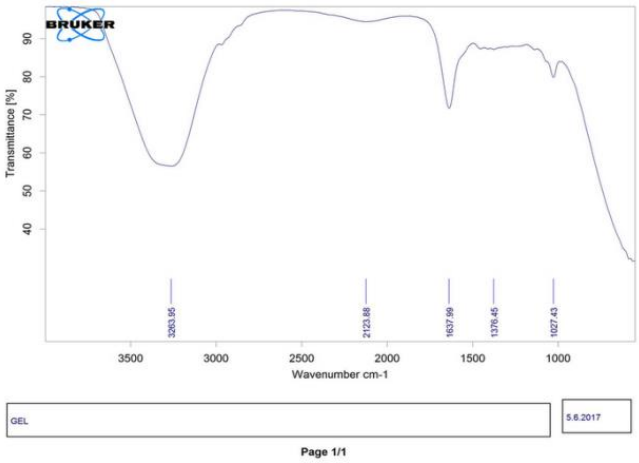

Figure 2: FTIR spectra of the *O. indicum* extract loaded herbal gel

Characterization of the Prepared Herbal Hydrogels:

The prepared *Oroxylum indicum* herbal topical gel was greenish in color. Results of the visual inspection of the prepared gel showed satisfactory physical properties with smooth homogenous texture, non-greasy and good homogeneity with absence of lumps and syneresis and the blank formulations showed with smooth texture, very good homogeneity and non-greasy physical properties. Both the plain gel and formulated hydrogel shows good extrudability.

pH values of the prepared hydrogels were in the range of 6.8-7 which is considered acceptable to avoid skin irritation upon application. Results are illustrated in table 2.

Good spreadability is one of the criteria for gel to meet ideal qualities. It is the term expressed to denote the extent of area to which gel readily spreads on application. Therapeutic efficacy of a gel formulation also depends on its spreading value [16]. The determined spreadability values indicate that the polymers used provided hydrogels which can spread by shearing force of low magnitude. It was observed that formulated hydrogel was having better spreadability compared to the blank gel as shown in the table 2.

Viscosity for blank gel, formulated gel and standard marketed gel was determined and all measurements were done in triplicate and the average reading is recorded at 25°C as shown in the table 2.

Table 2: Characteristics of O.indicum extract herbal topical gel

Formulation	pH at 25°C	Spreadability (gm^2cm/sec)	Extrudability	Viscosity (cP) at 25°C
Formulated hydrogel	6.9±0.67	22.66±0.09	Good	3315.72
Plain gel	6.7±0.32	21.05±0.02	Good	11456.86
Marketed gel	7.0±0.00	22.90±0.67	Very good	3417.92

O.indicum hydrogels which showed promising results were subjected to stability studies at refrigerator (4°C) and ambient room conditions (25°C) for 3 months. After storage for 3 months, hydrogels did not show any change in color, odor, pH, drug content, rheological properties and no phase separation occurred as shown in the table 3. This indicated that the *O. indicum* extract was stable in gels even after 3 months of short term storage and the gel formulations were physically and chemically stable.

Table 3 Stability studies of the Formulated hydrogel, Plain gel, and Marketed gel after 3 months

Formulation	pH at 25°C	Spreadability (gm^2cm/sec)	Extrudability	Viscosity (cP) at 25°C
Formulated hydrogel	6.8±0.67	22.66±0.09	Good	3315.72
Plain gel	6.6±0.32	21.05±0.02	Good	11456.86
Marketed gel	7.0±0.00	22.90±0.67	Very good	3417.92

In-Vivo Antibacterial Studies

The antibacterial potential of the formulated hydrogel was evaluated on infection induced Wister rats with the desired bacterial species, i.e. *S. aureus* (Figure 3) and *S. epidermidis* (Figure 4). The efficacy of the formulation containing the plant extract against skin disease was assessed manually by observing the infection area and body weight followed by bioburden study after applying the formulated gel topically once a day for three weeks. The animal study reveals that the hydrogel formulation containing the plant extract cures almost 60 % of the wound infected with *Staphylococcus aureus* and 70 % wound infected with *Staphylococcus epidermidis*. These observations were supported by the results observed with the bioburden study. The body weights of all the groups increased as shown in table 4 and 5. The increase in body weights in control and positive and negative control groups are significant. But the change in test group is very less.

Table 4: Body weight of Control, Test group and Positive control group of *S.aureus*

Groups	Body weight (g)		
	Initial	Final	% Increase
Negative Control	86.66±11.54	120.0±0.00	38.64
Test	120.00±17.32	127.5±38.89	6.25
Positive control	120±43.20	200.0±0.00	66.67

Table 5: Body weight of Control, Test group and Positive control group of *S.epidermidis*

Groups	Body weight (g)		
	Initial	Final	% Increase
Control	76.66±20.81	110.00±0.00	43.478
Test	110.00±17.32	130.00±0.00	18.2
Positive control	86.66±15.27	120.00±0.00	38.46

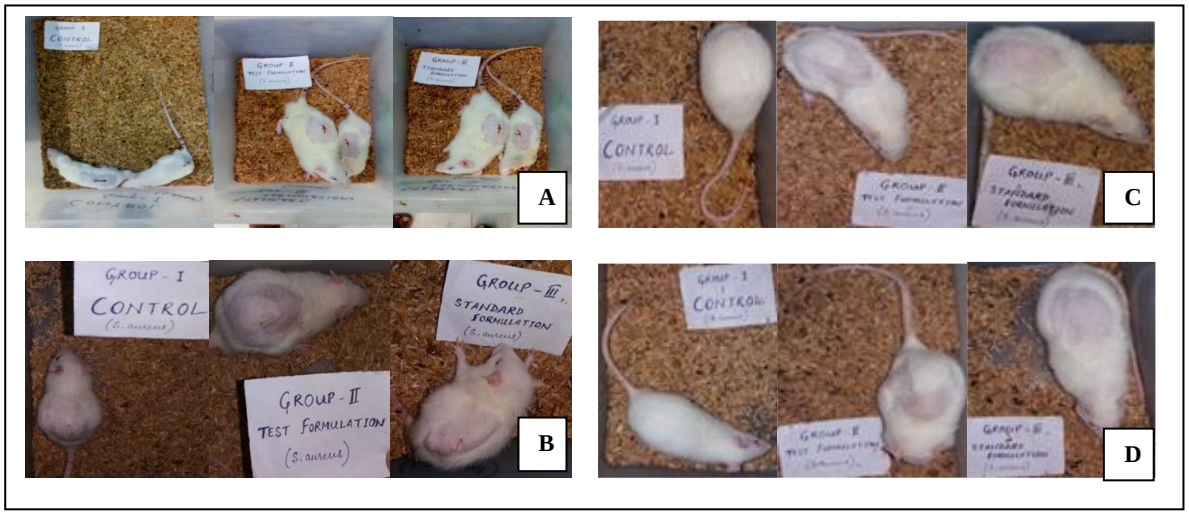

Figure 3: Treatment of skin infected with *Staphylococcus aureus* on different weeks (A-zero days, B-7th days, C- 14th days, D-21st days)

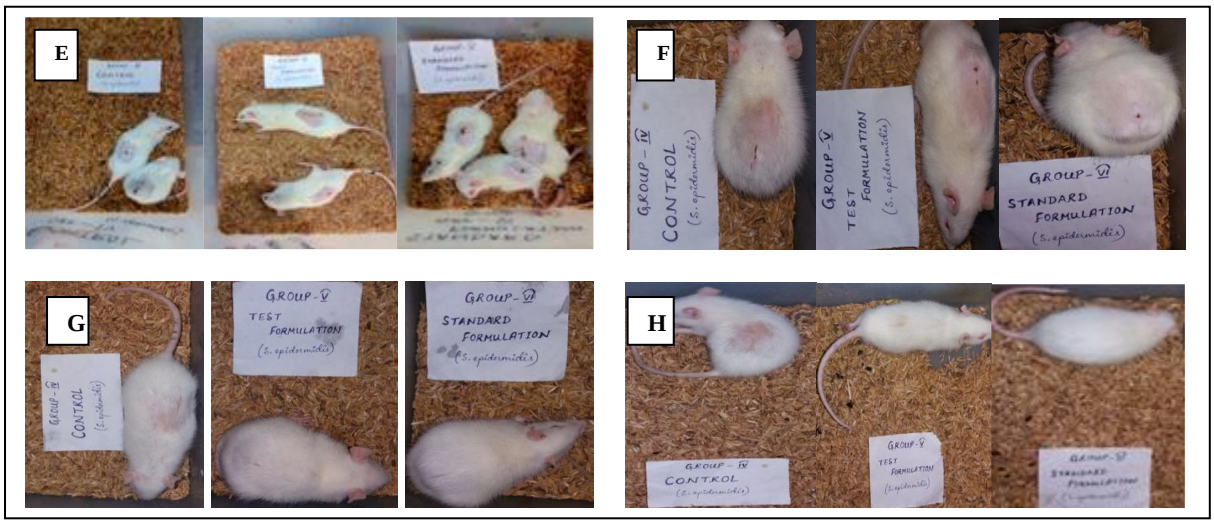

Figure 4: Treatment of skin infected with *Staphylococcus epidermidis*on different weeks (E-zero days, F-7th days, G- 14th days, H-21st days)

Bioburden Study

From the bioburden study it was seen that plates are in between 30 and 300 colonies. Fewer than 30 colonies are not acceptable for statistical reasons (too few may not be representative of the sample) and more than 300 colonies on a plate are likely to produce colonies too close to each other to be distinguished as distinct colony-forming units (CFUs). Thus from the results it is seen that each viable bacterial cell contain *Staphylococcus aureus* (Table 5) in the test group plate is lower than the control group and positive control group and each viable bacterial cell contain *Staphylococcus epidermidis* (Table 6)in test group plate is lower than the control group and the positive control group.

Table 5: Effect of *O.indicum* herbal gel formulation on bioburden study in Wister Albino rats (*Staphylococcus aureus)*

Groups	Bioburden (no. of bacteria/ml)
Control	2.91×10^8
Test	2.10×10^8
Positive control	2.27×10^8

Table 6: Effect of *O.indicum* herbal gel formulation on bioburden study in Wister Albino rats (*Staphylococcus epidermidis)*

Groups	Bioburden (no. of bacteria/ml)
Control	2.35×10^8
Test	2.18×10^8
Positive control	2.25×10^8

IV. CONCLUSION

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. The studies revealed that the developed single herbal formulation consisting of *Oroxylum indicum* extract was comparatively better and all the formulations were non-irritant. Topical application of *Oroxylum indicum* herbal gel prevented the wound infections on the skin of the treated animals. The formulations were carried out for physicochemical characteristics and pharmacological activity. The formulated gel was found to have optimum in terms of gel

consistency, spreadability, extrudability and viscosity and the pharmacological studies of the formulation revealed that the formulation has maximum antibacterial activity compared with the control group. It was found that antibacterial action of hydroalcoholic extract is an indication of presence of anti-pathogenic potential possessing flavonoids. Although the formulation is non-irritant and further pharmacological screening may implied to test and investigate the safety profile of formulated gel to treat various infections of skin. Hence our results suggest that *Oroxylum indicum* leaf extract has Antibacterial activity.

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