IJBPAS, February, 2024, 13(2): 502-512

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

FORMULATION AND EVALUATION OF HERBAL GEL FORMULATION FOR THE TREATMENT OF CELLULITIS

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Received 24th Dec. 2022; Revised 25th Jan. 2023; Accepted 8th May 2023; Available online 1st Feb. 2024

https://doi.org/10.31032/IJBPAS/2024/13.2.6934

ABSTRACT

The S. *aureus, Candida albicans, E . coli* and *Bacillus subtilis* are human pathogen which can cause bacterial skin infections. *Trigonella foenum graecum* Linn have antimicrobial, anti-inflammatory effect. The aim of this study is design, formulation, and evaluation of the herbal gel containing extracts of *Trigonella foenum graecum* Linn.

Materials and Methods:

In this experimental study after phytochemical and macroscopic evaluation of these medicinal herbs, the semisolid concentrated extracts were incorporated in gel bases. Gel formulations were prepared using carbopol 940 polymer and propylene glycol. The prepared formulations were subjected physicochemical evaluation, viscosity and *in vitro* drug release study.

Results:

Physical appearance, homogeneity, and consistency of all formulations were found good. Study of release profiles of formulations showed significant drug release. The antimicrobial activity results shown that the formulation containing greater concentration of extract shows greater zone inhibitions.

Conclusion:

The best formulation for treatment of bacterial skin infections should exhibit highest zone of inhibition show controlled release of drug. F_4 with the highest viscosity and mucoadhesion and the lowest release rate was considered as the best formulation.

Keywords: ***Trigonella foenum graecum Linn, Staphylococcus aureus, Candida albicans, Escherichia coli, Bacillus subtilis***

INTRODUCTION:

Cellulitis is a painful bacterial infection of the deeper layers of skin. It can start suddenly and become life threatening without prompt treatment. Mild cases involve a localized infection Trusted Source, with redness in one area. More serious cases involve a rapidly spreading infection that can lead to sepsis. The spread will depend, to some extent, on how strong the individual immune system is **[1]**. Cellulitis is an acute inflammatory condition of the dermis and subcutaneous tissue usually found complicating a wound, ulcer or dermatosis. Erysipelas, a superficial cellulitis with prominent lymphatic involvement, does have an indurated, raised border that demarcates it from normal skin **[2]**.

Cefazolin, a first-generation cephalosporin, nafcillin, an antistaphylococcal synthetic penicillin and ceftriaxone, a third-generation cephalosporin, are all initial treatment options. If methicillin-resistant *S. aureus* (MRSA) is suspected or the patient is highly allergic to penicillin, then vancomycin and linezolid are the drugs of choice and have similar cure rates **[3, 4]**. Initial treatment should be given by IV in the hospital if the inflammation is spreading rapidly, if there is a significant systemic response (chills and fever) or if there are complicating coexisting conditions like immunosuppression, neutropenia, cardiac failure or renal insufficiency **[5, 6]**. Diabetic foot infections require special care since they often involve multiple pathogens **[7]**. These cases usually present in such characteristic ways that anatomical location and the patient's medical and exposure history aid with diagnosis and guide appropriate antibiotic therapy **[8]**.

Although the gel formulation of *trigonella* seems to be highly useful and there is lack of literature on the formulation and evaluation of herbal gel **[9, 10]**. Therefore, in the present work, it is planned to prepare and evaluate herbal gels using aqueous and ethanolic extracts of *Trigonella foenum graecum Linn*. Seed. Topical preparations give its action directly at the site. The formulated herbal gel exhibits anti – inflammatory activity and antimicrobial activity. Hence for local inflammation or bacterial infection in the body, the topical application of herbal gel may be useful which also reduces the side-effects associated with oral therapy **[9]**.

MATERIALS AND METHODS:

Trigonella foenum graecum Linn seed were collected from local market Latur, carbopol 940, Methyl paraben, Propyl paraben, propylene glycol and triethanolamine were purchased from S. D. Fine. Chem. Pvt. Limited, Mumbai.

EXPERIMENTAL AND RESULTS:

ED-XRF analysis: Element Determination: The main objective of ED-XRF analysis is to determine the concentration of trace elements in selected seeds of *Trigonella foenum graecum* Linn which plays an important for the treatment of dermal diseases. The trace element analysis was carried out in *Trigonella foenum-graecum,* using ED-X-ray Florescence (XRF) technique. The experiments were carried out using 3MV pelletron accelerator. The elements K, Ca, Cr, Mn, Fe, Cu, Zn, Rb, Sr and Pb were identified in the sample **[11-13]**.

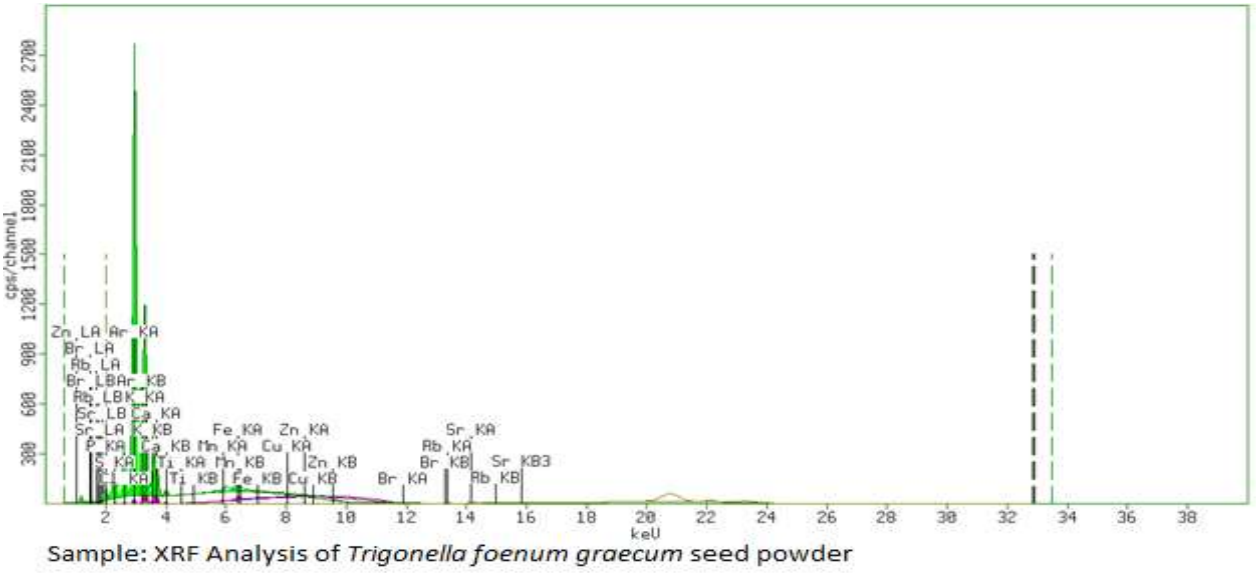


Figure 1

Table 1:

Elements	*Trigonella foenum graecum* Linn (seed)	Vitex nigundo Linn	*Argemone mexicana* Linn	*Azardirachta indica* Linn
Fe	**2.022%**	**5.387%**	**2.467%**	**1.977%**
Mn	**0.466%**	**0.561%**	**0.225%**	**0.371%**
Zn	**0.411%**	**0.204%**	**0.049%**	**0.047%**
Cu	**0.162%**	**0.054%**	**0.030%**	**0.024%**
V	**0.00%**	**0.051%**	**0.076%**	**0.107%**
Si	**0.00%**	**0.227%**	**0.000%**	**0.213%**
P	**4.669%**	**1.628%**	**1.195%**	**0.966%**
S	**5.184%**	**1.910%**	**1.619%**	**1.733%**
Cl	**8.138%**	**2.611%**	**3.971%**	**5.733%**
K	**56.797%**	**31.975%**	**35.399%**	**20.825%**
Ca	**21.827%**	**54.418%**	**54.450%**	**67.265%**
Ti	**0.176%**	**0.842%**	**0.385%**	**0.391%**
Br	**0.033%**	**0.000%**	**0.022%**	**0.186%**
Rb	**0.074%**	**0.019%**	**0.040%**	**0.007%**
Sr	**0.042%**	**0.07%**	**0.056%**	**0.154%**

Extraction of plant Material:

The air dried powdered *Trigonella foenum graecum seed* (500g) were extracted with ethanol (95%v/v) by maceration and concentrated on water bath to get ethanol extract. The air dried powdered *Trigonella foenum graecum seed* (500g) were extracted with distilled water by maceration and concentrated on water bath to get aqueous extract **[14, 15]**.

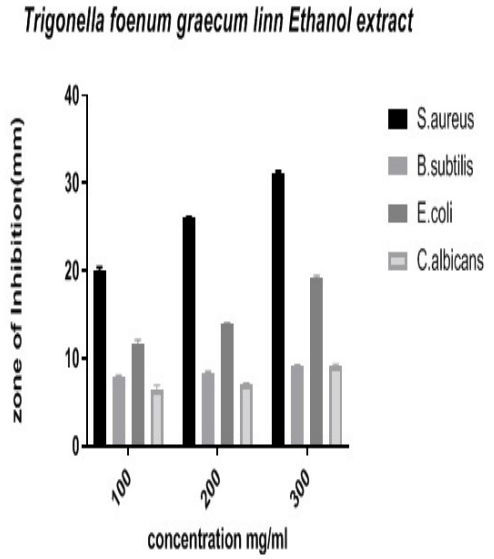
Antimicrobial activity of aqueous and ethanolic extracts of *Trigonella foenum graecum Linn:*

The antibacterial activity of all plants was determined against *S.aureus, Candida albicans, E.coli* and *Bacillus subtilis* at Three different concentrations 100 mg/ml, 200mg/ml, of each aqueous and ethanol extract by the agar well diffusion method. Microorganisms used for the study were obtained from the National collection of industrial microorganisms NCIM, Pune Maharashtra, India. The test organisms included *Candida albicans* MTCC 183, *E. coli* MTCC 40, *B.subtilis* MTCC 121, *S.aureus* ATCC 2079 which were used in the present study. The bacteria were grown in nutrient broth (Himedia, M002) at 37±1ºC and maintained on nutrient agar at 40^0C and *C.albicans* on potato dextrose agar media **[11, 12]**.

Agar well diffusion method. *Trigonella foenum graecum* Linn extract were tested against *Candida albicans, E.coli, B.subtilis,* and *S.aureus* which are the common bacteria causing skin diseases by agar well diffusion method. The extracts were prepared using 10% DMSO as a solvent. *E. coli, B.subtilis, S.aureus* was incubated on nutrient agar plates at 37°C for 48 hrs. Under aerobic conditions. The antimicrobial activity of these plant extract (Aqueous and Ethanol) was determined against *Candida albicans, E.coli, B.subtilis*, *S.aureus* at three different concentrations of each extract by the agar well diffusion method **[13-16, 24]**.

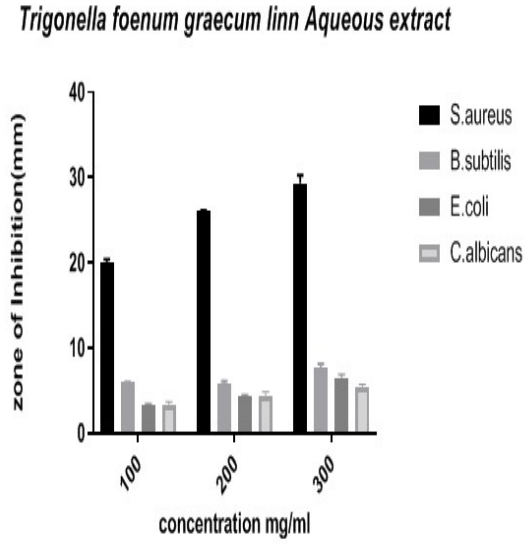
Table 2

Sr.no	*Trigonella foenum graecum* Linn extract	Conc. (mg/ml)	Zone of Inhibition in mm			
			E. coli	*S.aureus*	*B.subtilis*	*Candida albicans*
1	*Trigonella foenum graecum* Linn Aqueous extract	100	3.0+0.1	28.0+0.01	6.1+0.01	3.1+0.02
		200	4.0+0.3	32.0+0.03	6.5+0.02	4.2+0.03
		300	6.0+0.4	30.0+0.07	8.0+0.07	5.1+0.07
2	*Trigonella foenum graecum* Linn Ethanol extract	100	12.0+0.07	20.5+0.04	8.0+0.02	6.0+0.03
		200	14.0+0.08	26.0+0.03	8.0+0.04	7.2+01
		300	19.32+0.2	31.3+0.06	9.0+0.07	9.0+0.03



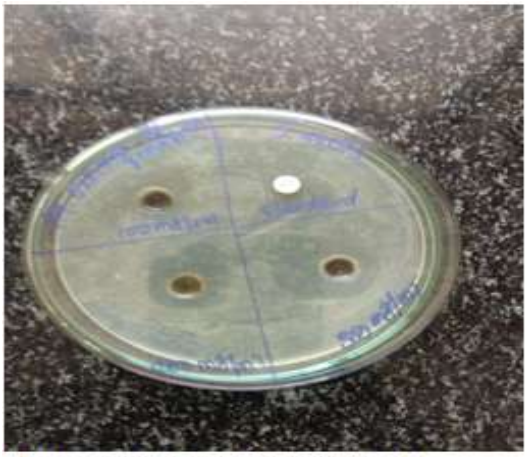
Antimicrobial activity of Trigonella foenum graecum Linn Ethanol extract

Figure 2



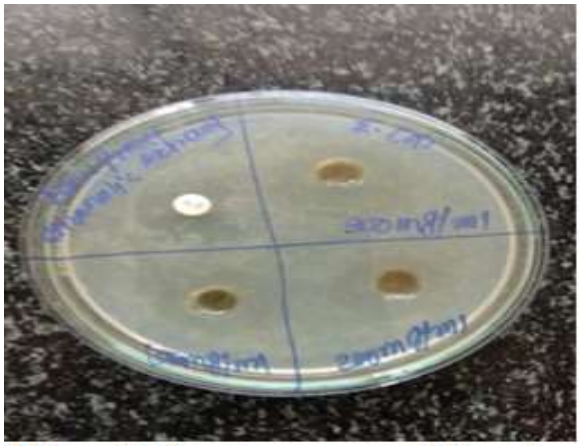
Antimicrobial activity of Trigonella foenum graecum linn aqueous extract

Figure 3



Trigonella foenum graecum Aq. extract

Figure 4



Trigonella foenum graecum Ethanol extract

Figure 5

Acute dermal Toxicity study:

The study procedures involving the treatment and handling of animals were approved by the Institutional animal ethical committee of Channabasweshwar Pharmacy College, Latur with Ref.No. CPCSEA/CBPL/AH/52 Dorsal hairs at the back of the rats were clipped off one day prior to the commencement of the study.

results showed no noticeable signs of acute toxicity and lack of death at doses of 2000 mg/kg body weight **[17, 18]**.

Results of acute toxicity study:

The acute toxic study results of selected plant extract showed no noticeable signs of acute toxicity and lack of death at dose of 2000 mg/kg body weight. Hence, lethal dose (LD50)

for topical route of selected plant extract was estimated to be higher than 2000 mg/kg.

Table 3

parameters	Cage side observations	
	Control group	Treated group
Behaviour pattern	Normal	Normal
Gait	Normal	Normal
Skin	Normal	Normal
Condition of the fur	Normal	Normal
Tremors	NIL	NIL
Convulsions	NIL	NIL
Eye-dullness	Normal	Normal
Salivation	Normal	Normal

Formulation of Herbal Gel: [19, 20]

Table 4: Formulations of *Trigonella foenum graecum* Linn from aqueous extract:

Ingredients	Formulation code Quantity taken per 100 (in grams)		
	F1 TA	F2 TA	F3 TA
Carbopol-940	1%	1%	1%
Propylene glycol 400	4%	4%	4%
Triethanolamine	q.s	q.s	q.s
Dist. Water	q.s 100	q.s 100	q.s 100
Methyl paraben	0.2%	0.2%	0.2%
Propyl paraben	0.02%	0.02%	0.02%
Trigonella foenum graecum Aqueous Extract	2%	3%	4%

Table 5: Formulations of *Trigonella foenum graecum* Linn from aqueous extract:

Ingredients	Formulation code Quantity taken per 100 (in grams)		
	F1 E	F2 E	F3 E
Carbopol-940	1%	1%	1%
Propylene glycol 400	4%	4%	4%
Triethanolamine	q.s	q.s	q.s
Dist. Water	q.s 100	q.s 100	q.s 100
Methyl paraben	0.2%	0.2%	0.2%
Propyl paraben	0.02%	0.02%	0.02%
Trigonella foenum graecum ethanol Extract	2%	3%	4%

Table 6: Antimicrobial activity of *Trigonella foenum graecum* Linn formulations from Aqueous extract

Formulation	Concentration (mg/ml)	Zone of inhibitions in mm			
		S.aureus	*E.coli*	*B.subtilis*	*C.albicans*
F1 A (2%)	100mg/ml	16.7+0.04	3.1+0.05	4.5+0.02	3.5+0.02
	200mg/ml	17.8+0.09	3.9+0.06	5.9+0.04	3.8+0.07
	300mg/ml	18.1+0.03	4.2+0.02	6.2+0.04	4.1+0.02
F2 A (3%)	100mg/ml	19.0+0.01	4.3+0.06	4.7+0.07	2.7+0.01
	200mg/ml	21.6+0.04	4.9+0.2	5.7+0.6	3.3+0.04
	300mg/ml	25.2+0.06	5.2+0.07	6.5+0.06	4.5+0.09
F3 A (4%)	100mg/ml	27.9+0.04	5.5+0.01	6.8+0.04	4.0+0.05
	200mg/ml	28.6+0.07	6.9+0.02	7.3+0.02	5.07+0.01
	300mg/ml	31.2+0.08	7.0+0.01	8.3+0.06	6.2+0.07

Table 7: Antimicrobial activity of *Trigonella foenum graecum* formulations from Ethanol extract:

Formulation	Concentration mg/ml	Zone of inhibitions in mm (*Trigonella foenum graecum* Linn)			
		S.aureus	*E. coli*	*B.subtilis*	*C.albicans*
F1 E (2%)	100mg/ml	18.7+0.04	9.5+0.05	6.5+0.03	5.5+0.03
	200mg/ml	19.8+0.09	10.9+0.06	6.9+0.05	6.8+0.05
	300mg/ml	21.4+0.05	11.3+0.02	7.2+0.06	7.5+0.04
F2 E (3%)	100mg/ml	23.0+0.01	12.3+0.05	6.7+0.08	5.7+0.02
	200mg/ml	25.6+0.04	13.9+0.31	7.7+0.5	6.3+0.03
	300mg/ml	26.0+0.04	14.1+0.06	8.1+0.01	8.1+0.07
F3 E (4%)	100mg/ml	29.9+0.04	15.5+0.02	6.8+0.01	7.0+0.05
	200mg/ml	30.6+0.07	17.9+0.04	7.3+0.03	8.07+0.01
	300mg/ml	32.4+0.05	18.32+0.01	9.7+0.06	9.8+0.04

Trigonella foenum graecum Ethanol extract formulations

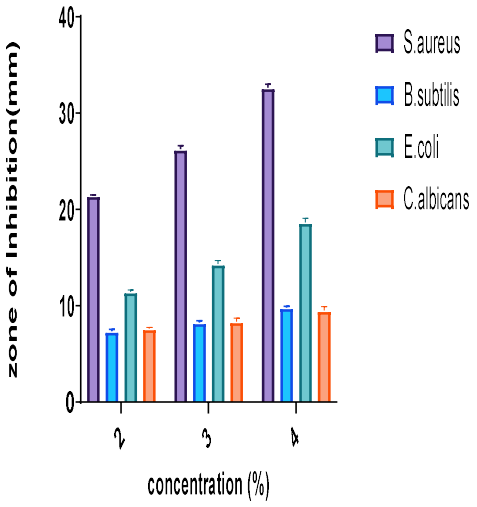


Figure 6

Trigonella foenum graecum Aqueous extract formulations

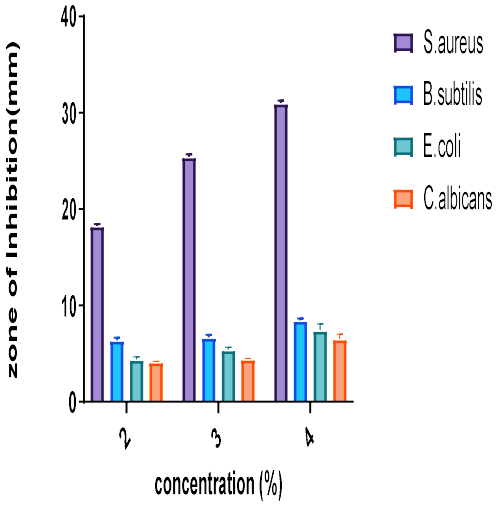


Figure 7

Skin Irritancy Test of Optimized Formulation:

The primary skin irritation test will be performed on rats and weighing about 150-200 gm. Animals divided into two groups. (n=2)

Group I- Control Rat without treatment

Group II- First site for Gel formulation and second site for blank gel base (without extract) applied. The animals were observed for 0, 24, 48, 72 hours for any signs of change in colour, changes in skin morphology, edema and erythema. There are no signs of skin irritation to rat, no change in skin morphology, edema and erythema **[21-23]**.

Anti-inflammatory activity of Optimized Formulation:

Carrageenan-induced rat paw edema:

All animal procedures were followed in three groups (Control, Test and Standard) of six animals each. The animals were maintained on

standard animal feed and had free access to water. Approximately 50uL of 1% suspension of carrageenan in saline were prepared 1 h before each experiment and injected into the plantar side of right hind paw of rat. Herbal gel applied to the plantar surface of the hind paw by gentle rubbing with the index finger. Rat of the control groups received the plain gel base and 0.2 g 1% Diclofenac gel applied in the same way use as a standard. Drugs or placebo applied 1h before the carrageenan injection.

group I (Negative control): carrageenan+ Gel **Base** (50uL of 1% carrageenan to the sub plantar region) and saline water 0.1 mL/kg bw)

***group II (positive control):** carrageenan + **Diclofenac** Gel (0.2g/kg BW)

***group III (treated group):** carrageenan + herbal gel formulation.

Table 8:

Time/Treatment	Control Group	Test Group		Std Group	
	Mean ± SD	Mean ± SD	% Of inhibition	Mean ± SD	% Of inhibition
0 min	1.22±0.0684	1.22±0.1367	15.79	1.21±0.0817	5.26
After 30 min	1.31±0.1126	1.1±0.1674ns	13.56	1.1±0.0817**	27.12
After 1 hours	1.32±0.3734	1.29±0.0822**	39.16	1.32±0.0822**	39.16
After 2 hours	1.36±0.1084	1.15±0.1761*	24.44	1.16±0.1674*	20.00
After 3 hours	1.31±0.045	1.19±0.1761**	24.22	1.22±0.1242*	19.02
After 4 hours	1.35±0.1509	1.18±0.3387ns	16.34	1.19±0.3205ns	18.95

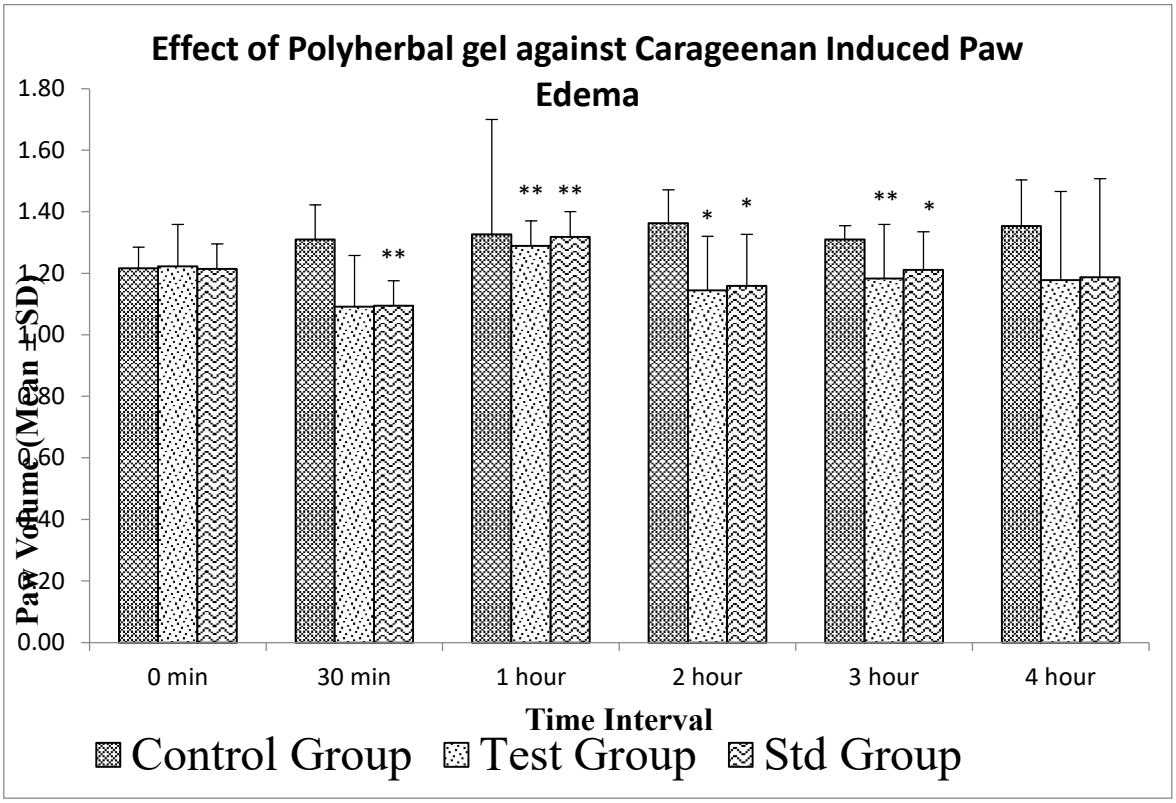


Figure 8: Anti-inflammatory activity of optimized gel formulation

CONCLUSION:

The phytochemical analysis of *Trigonella foenum graecum* Linn aqueous and ethanolic extract shows presence of various important phytoconstituents aqueous and ethanolic plant extract subjected for in-vitro antimicrobial activity at different concentration 100mg/ml, 200mg/ml, 300mg/ml against *S.aureus*, *E.coli*, *Candida albicans* and Bacillus Subtilis and results shown that ethanol extract of plant shows greater results recorded in the form of zone of inhibitions at concentration of 300 mg/ml. On the basis of antimicrobial activity of plant extract different concentration of extract 2%, 3%, 4% incorporated into herbal gel formulation and subjected for in-vitro antimicrobial activity against *S.aureus*, *E.coli*, *Candida albicans* and *B.subtilis*, shows greater results at concentration of 4% ethanol extract. Finally, herbal gel (ethanol) was prepared and subjected for antimicrobial activity, on the basis of zone of inhibitions and physicochemical parameters, the herbal gel consisting 4% ethanol extract were optimized. Optimized formulation was screened for Skin Irritancy test and In-Vivo anti-inflammatory study by using carrageenan induced rat paw edema method and shows greater results. The stability study results found that the herbal gel was stable for three months. There is a great potential in the delivery of selected plant extract in the form of herbal gel for the treatment of bacterial skin disease like cellulitis. Therefore, the given herbal gel formulations contain a combination of antimicrobial and anti-inflammatory properties, which proved as promising anti-microbial agent and anti-inflammatory agent for bacterial skin diseases. However long term clinical trials are needed to access its efficacy and safety.

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