FORMULATION AND CHARACTERIZATION OF *ZIZIPHUS MAURITIANA* CONTAINING HERBAL GEL FOR EFFECTIVE ANTIMICROBIAL ACTIVITY

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Abstract

The development of an herbal gel containing *Ziziphus mauritiana* was investigated for its potential antimicrobial activity and physicochemical properties. The gel formulations were characterized for homogeneity, spreadability, extrudability, pH, viscosity, drug content, and cumulative drug release. The antimicrobial efficacy of the optimized formulation was tested against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Results showed that all formulations exhibited good homogeneity and favorable spreadability, with the optimized gel (F4) displaying the best properties in terms of physical appearance, viscosity, and drug content (99.45%). The gel demonstrated sustained drug release, with 96.65% of the active ingredient released at 10 hours. Antimicrobial testing revealed that the formulation exhibited dose-dependent inhibition against both microbial strains, with significant zones of inhibition observed. The stability studies confirmed that the optimized gel maintained its drug content and physical appearance over three months, under both refrigerated and room temperature conditions. These findings suggest that *Ziziphus mauritiana*-based herbal gel is an effective topical formulation for treating skin infections, combining traditional herbal medicine with modern pharmaceutical technology.

Keywords

Ziziphus mauritiana, herbal gel, antimicrobial activity, *Staphylococcus aureus*, *Klebsiella pneumoniae*, drug release, formulation, gel properties, stability, antimicrobial efficacy.

Introduction

Herbal gels are innovative topical formulations that incorporate the therapeutic properties of natural plant extracts, making them a popular choice for skincare, pain relief, and overall wellness. These gels offer a natural alternative to synthetic products by delivering active botanical ingredients directly to the skin, providing targeted treatment with minimal side effects (Das *et al.*, 2010). Unlike creams or ointments, herbal gels have a light, non-greasy texture that allows for quick absorption, making them ideal for those who prefer a refreshing application. The cooling sensation often associated with gels enhances their appeal, particularly in soothing irritated or inflamed skin. Herbal gels are versatile, being

used in various contexts such as treating acne, moisturizing dry skin, treating skin disease. This unique blend of traditional herbal knowledge and modern formulation techniques makes herbal gels an effective and convenient way to harness the healing powers of nature (Dixit *et al.*, 2013).

Herbal gels formulated as antimicrobial agents are an effective and natural approach to combating harmful microorganisms on the skin. These gels are infused with plant extracts known for their antimicrobial properties, offering a natural alternative to synthetic antiseptics and antibiotics (Sharma *et al.*, 2012). The active ingredients in these herbal gels, such as phenol and flavonoids, work synergistically to inhibit the growth of bacteria, fungi, and other pathogens (Das et al., 2011).

The gel base allows for easy and targeted application, ensuring that the antimicrobial agents are delivered directly to the affected area. Additionally, the quick-absorbing and non-greasy nature of gels makes them convenient to use, leaving the skin feeling clean and refreshed without residue. Herbal antimicrobial gels are particularly useful in treating minor cuts, wounds, and skin infections, where they can help prevent infection and promote faster healing (Rathod and Mehta, 2015).

This study aims to formulate and characterize a gel containing *Ziziphus mauritiana* extract, evaluating its physical properties such as homogeneity, spreadability, and extrudability, as well as its antimicrobial efficacy against selected microbial strains. The formulation's stability, drug release profile, and antimicrobial activity are crucial factors that will determine its potential as an effective therapeutic agent in treating skin infections.

Material and Methods

Preparation of topical gel

The incorporation of the herbal *Ziziphus mauritiana* loaded gel (equivalent to 1%) into gels was achieved by slow mechanical mixing at 25 rpm (REMI type BS stirrer) for 10 minutes. The optimized formulation was incorporated into three different Carbapol 934 and Carbapol 940 gel concentration 0.5, 1 and 2% w/w (Goyal *et al.*, 2011) as shown in table 1.

Table 1: Composition of different gel base of *Ziziphus mauritiana* extract

S. No.	F. Code	Extract (%)	Carbapol 934 (%)	Carbapol 940 (%)	TEA (ml)	Methylparaben	Propylparaben
1.	F1	1	0.5	-	0.2	20	80

2.	F2	1	1	-	0.2	20	80
3.	F3	1	2	-	0.2	20	80
4.	F4	1	-	0.5	0.2	20	80
5.	F5	1	-	1	0.2	20	80
6.	F6	1	-	2	0.2	20	80

Evaluation of herbal gel

Physical characteristic

The physical characteristic was checked for gel formulations (homogeneity and texture) and observations were shown in Table 8.3 (Rajasree *et al.*, 2012).

Determination of pH

The pH of the gel was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. The measurements of pH of each formulation were replicated two times (Pandey *et al.*, 2010).

Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually and observations were shown in Table 8.3.

Extrudability study

The gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked in table 8.3 (Yamini and Onesimus, 2013).

Assay

Weight equivalent to 10 mg of gel dissolved in 5 ml methanol in 10 ml volumetric flask, sonicate it for 10 min and volume make up to 10 ml and dilute suitably to 10µg/ml and take the absorbance at 238 nm and calculate using calibration curve of linearity (Niyogi *et al.*, 2012).

Spreadability

Two glass slides of standard dimensions (6×2) were selected. The gel formulation whose spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cms along the slide. 100 grams of weight was placed up on the upper slide so that the gel formulation between the two slides was traced uniformly to form a thin

layer. The weight was removed and the excess of the gel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied 50 with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cms and separate away from lower slide under the direction of the weight was noted.

The experiment was repeated and the average of 6 such determinations was calculated for each gel formulation (Vandana and Pawar, 2019):

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)

m = weight tied to the upper slide (20 grams)

l= length of glass slide (6cms).

t = time taken is seconds.

Viscosity

The measurement of viscosity of the prepared gel was done using Brookfield digital Viscometer. The viscosity was measured using spindle no. 6 at 10 rpm and 25^0C. The sufficient quantity of gel was filled in appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow to dip the spindle of the Viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25±/1^0C) before the measurements.

***In-vitro* drug release studies using the semipermeable membrane**

The semipermeable membrane approximately 25cm x 2cm was taken and washed in the running water. It was then soaked in distilled water for 24 hours, before used for diffusion studies to remove glycerin present on it and was mounted on the diffusion cell for further studies. The prepared herbal gel was evaluated for *in vitro* drug release. The drug release studies were carried out using modified franz diffusion cell. The dissolution study was carried out in 24 ml dissolution medium which was stirred at 50 rpm maintained at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 10ml by PBS (pH 7.4). The samples withdrawn were assayed spectrophotometrically at 285nm for extract and using UV visible

spectrophotometer (Deepak and Prashanth, 2013). The release of extract from gel was calculated with the help of Standard curve of extract.

Stability studies

Stability study data was revealed that the optimized formulation (F-4) stable after 3 month of storage at 4ºC while at 25-28±2°C, the formulation was found unstable. Stability of formulation was observed on the basis of % drug remain, average vesicles size and physical appearance (Ajinkya and Manjusha, 2016).

***In vitro* antimicrobial activity of optimized gel formulation F4**

The well diffusion method was used to determine the antimicrobial activity of the gel prepared from of *Ziziphus mauritiana* using standard procedure (Bauer, 1966). There were 3 concentration used which are 25, 50 and 100 mg/ml in studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37ºC for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

Results and discussion

The results of the formulation and characterization of the *Ziziphus mauritiana*-containing herbal gel indicate that the gel exhibits promising physical and antimicrobial properties. The homogeneity and texture of all formulations (F1 to F6) were rated as "+++", indicating that the gels were smooth and consistent, which is essential for uniform distribution of the active ingredient. The spreadability, which ranges from 11.45 gm.cm/sec. (F6) to 15.52 gm.cm/sec. (F1), suggests that F1, F2, and F3 are particularly suitable for easy application, as they allow for smoother spread on the skin. Extrudability, which is important for the ease of use, was rated "+++" for F1, F2, and F3, while F4, F5, and F6 received a "++" rating. This means that the first three formulations are easier to dispense from the tube. Additionally, all formulations demonstrated good washability, indicating that they can be easily removed from the skin after use.

In terms of pH, the gel formulations maintained skin-friendly pH values ranging from 6.74 to 6.95, which is ideal for ensuring skin compatibility. The viscosity, which ranged from 3065 cps in F6 to 3458 cps in F1, suggests that the formulations with higher viscosity may have better gel consistency and longer retention on the skin, potentially enhancing the drug's localized action. The drug content in the formulations was found to be high, with F4

having the highest drug content at 99.45%. Although there were slight variations in drug content across the formulations, the overall content remained close to the desired percentage, indicating good formulation stability.

The cumulative drug release data for the optimized formulation (F4) showed a steady release of the active ingredient over time. At 10 hours, 96.65% of the drug was released, which suggests that the gel formulation is capable of providing sustained drug delivery, which is beneficial for prolonged antimicrobial action. This sustained release profile enhances the gel's potential for effective treatment over extended periods, making it suitable for use in topical applications.

Stability studies revealed that the formulation (F4) remained physically stable and effective over time. Although some minor decrease in drug content was observed over three months (from 99.45% to 96.65%), the gel maintained a normal appearance and showed no significant changes in its physical properties. These findings suggest that the herbal gel is stable both at refrigerated (4°C) and room temperature (25–28°C), ensuring its shelf-life is adequate for practical use.

The antimicrobial activity of the optimized gel (F4) was evaluated against *Staphylococcus aureus* and *Klebsiella pneumoniae*, with the results showing a dose-dependent increase in the zone of inhibition. At 100 mg/ml, the gel demonstrated a 25 mm zone of inhibition against *S. aureus* and a 20 mm zone against *K. pneumoniae*. This confirms the gel's strong antimicrobial potential, which is crucial for its therapeutic applications in treating skin infections caused by these pathogens.

Table 2: Results of Homogeneity, Extrudability, Spreadability of gel formulation

Code	Homogeneity and Texture	Spreadability (gm.cm/sec.)	Extrudability	Washability
F1	+++	15.52	+++	Good
F2	+++	14.65	+++	Good
F3	+++	13.22	+++	Good
F4	+++	14.98	++	Good
F5	+++	13.22	++	Good
F6	+++	11.45	++	Good

+++ Good ++ Average

Table 3: Results of pH, viscosity and % drug content

Code	pH	Viscosity (cps)	% Drug content
F1	6.85±0.15	3458±15	97.74±0.23
F2	6.95±0.32	3365±13	96.65±0.14
F3	6.75±0.25	3215±23	98.85±0.32
F4	6.85±0.14	3298±24	99.45±0.20
F5	6.82±0.36	3145±26	94.45±0.16
F6	6.74±0.36	3065±21	93.32±0.32

Table 4: Cumulative % drug release of extract from optimized gel formulation F4

S. No.	Time (hrs)	% Cumulative drug release
1	0.5	33.32±0.25
2	1	48.85±0.35
3	2	55.65±0.18
4	4	68.85±0.22
5	6	79.45±0.65
6	8	85.56±0.14
8	10	96.65±0.22

Table 5: Characterization of Optimized formulation of herbal gel

Characteristic	Time (Month)					
	1 Month		2 Month		3 Month	
Temperature	4.0 ±0. 2°C	25-28±2°C	4.0 ±0.2°C	25-28±2°C	4.0 ±0.2°C	25-28±2°C
Percentage Drug content	99.45	98.85	99.25	97.65	99.05	96.65
Physical Appearance	Normal	High turbid	Normal	Normal	Normal	Normal

Table 6: Antimicrobial activity of optimized gel formulation F4 against selected microbes

Sr. No.	Microbes	Zone of Inhibition (mm)		
		25 mg/ml	50 mg/ml	100 mg/ml
1.	*Staphylococcus aureus*	16 ± 0.57	21 ± 0.47	25 ± 0.86
2.	*Klebsiella pneumoniae*	12 ± 0.94	15 ± 0.5	20 ± 0.74

***Average of three determination, Mean ± SD**

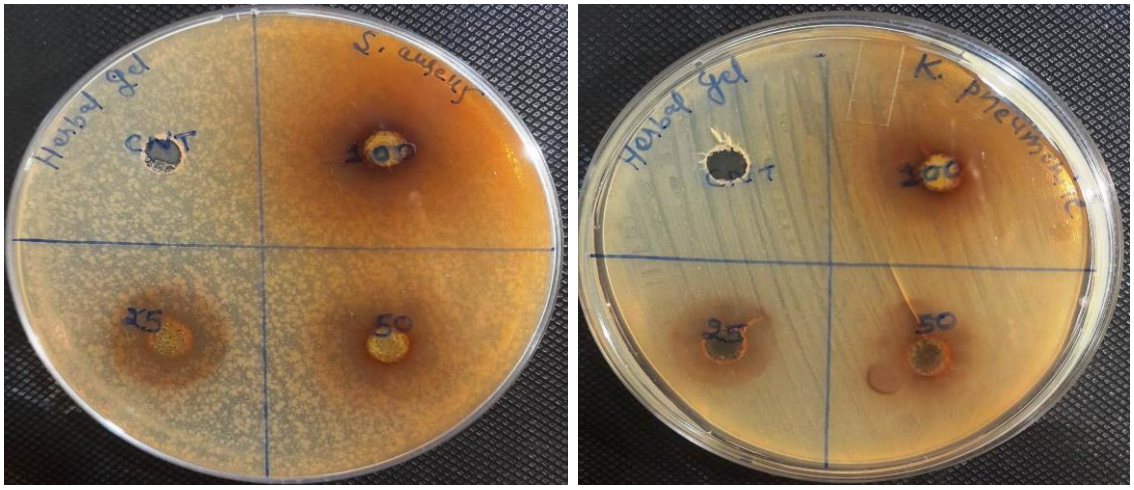

Figure 1: Photoplate of antimicrobial activity of optimized gel formulation F4 against selected microbes

Conclusion

In conclusion, the optimized herbal gel formulation F4 demonstrated significant antimicrobial activity against both *Staphylococcus aureus* and *Klebsiella pneumoniae*, with a clear dose-dependent increase in effectiveness. The gel was particularly effective against *S. aureus*, showing strong inhibitory effects even at lower concentrations, while also exhibiting considerable activity against *K. pneumoniae*. These results highlight the potential of herbal gels as a natural and effective antimicrobial agent, capable of combating both Gram-positive and Gram-negative bacteria. The study underscores the therapeutic value of incorporating plant-based ingredients into topical formulations, offering a promising alternative to synthetic antimicrobial treatments, especially in the context of skin infections.

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