



FORMULATION AND EVALUATION OF POLYHERBAL TOPICAL GEL FOR THE TREATMENT OF DERMATOLOGICAL INFECTIONS

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ABSTRACT

Background: Pathogenic bacterial species of humans with polymicrobial infections frequently show synergistic interactions that increase colonization and survivability. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the two most popularly known skin infections causing pathogenic bacteria, monoculture infection of particular species are less chronic rather than their co-infection of interspecies. Skin infections are generally cured by topical gel as a first-line treatment because of their non-sticky texture, good stability and aesthetic value.

Methods: Medicinal oils were selected and incorporated into a gel base to evaluate their physicochemical properties such as pH, viscosity, spreadability and extrudability and evaluate antibacterial potential against selected bacterial species.

Results: The physicochemical evaluation observed for three formulations could be easily washable; F3 shows high viscosity and extrudability while F2 shows high spreadability. It is observed that the F3 formulation shows high antibacterial potential.

Conclusion: This study was to formulate a polyherbal topical gel containing Coconut oil, Neem leaves ethanolic extract and Tea tree oil to treat skin related fetal infections causing by *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which is as effective as marketed conventional gel.

Keywords: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Extrudability, Spreadability, Viscosity

INTRODUCTION:

Bacteria are omnipresent and maintain a symbiotic correlation with the environment; it could be positive or negative. Positive correlation maintains a healthy environment while negative correlation comprises harmful impacts on public health, leading to severe infections [1]. The infection ranged from mild pyoderma to serious life-threatening COVID-19. Usually, bacterial infections are more easily curable than any other microbial infections. In the recent past scenario, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are society's most common skin infections; both species are notorious and show high resistance against the antibiotic [2].

Medical practitioners have used various formulations to treat dermatological infections that vary from solid to liquid dosage. Among all these, topical agent gel is the most accepted formulation due to its high spreading property and easy to use [3]. A gel is a colloid having 99% wt. liquid, which is restrained by surface tension and a macromolecular network of fibers made by a small amount of a gelatin substance. Despite many advantages of gels, a restriction is the delivery of hydrophobic drugs. The preparation of gels has increased in both the cosmetics and pharmaceutical industries [4].

As the world modernized, people have increased the charms to herbal medicine. These herbal medicines may flaunt underlying or no side effects on the body like synthetic drugs. Researchers have been going through the analysis of herbal components then isolated and extracted the healing properties [5]. The chemical moiety responsible for efficacy was synthesized in modern laboratories to be incorporated into modern medicines. Now the manufacturing units of herbal drugs are marketed herbal products as pills, tablets and capsules form in large amounts, these preparations were generally polyherbs. The formulation of Polyherbs must include two or more herbal combinations; this kind of formulation is more potent and effective against various human diseases. Chawanprash and Triphala is a well-known poly combination example of Charka Samhita; both are more effective in combination form than the single one [6].

Several researchers reported antibacterial activity of Neem leaves, Tea tree oil, and Coconut oil in their study, still no availability of dermatological gel in this polyherbal formulation available in market. Therefore, in this regard, the present study is an attempt to formulate a polyherbal topical gel containing extract of neem leaves, coconut

oil, and tea tree oil and evaluating its antimicrobial potency.

METHODOLOGY

The present study was conducted between June 2019 to June 2020 in the Dept. of Pharmaceutical Science, Department of Pharmaceutical Sciences, Sir J. C. Bose Technical campus, Bhimtal, Kumaun University Nainital-263136, Uttarakhand, India. It is an educational institute that focuses on the design and synthesis of potential drugs, as well as their evaluation in biological system. Present study focused on the formulation of polyherbal gel against *Pseudomonas*

aeruginosa and *Staphylococcus aureus* co-infections, both are most common cause of chronic skin infection. Evidence suggests that *P. aeruginosa* and *S. aureus* co-infection are more virulent and results are more worse than single infections, and both species are notorious for their resistance to antimicrobials. Antibacterial results of polyherbal gel were well documented Tables and figures.

1: Preparation of Gel

Formulation F1, F2, and F3 were prepared which comprised ethanolic extract of Neem,

Tea Tree Oil, and Coconut oil in different concentration with Carbopol 934, propylene glycol 400 and ethanol, methylparaben, propylparaben, EDTA, and triethanolamine were used to prepare 100g of the gel by adding a sufficient quantity of distilled water. The water required for these formulations was divided into two parts. In one part particular amount of extract was dissolved and to this calculated quantity of propylene glycol 400 and ethanol were added and in other parts, Carbopol 934 was dissolved and to this solution methylparaben, propylparaben, and EDTA was added. Both of the solutions were mixed in a beaker and triethanolamine was added to the mixture drop wise to obtain the gel consistency. It was stirred by using a propeller for 2 hrs at 500rpm to obtain a homogenous gel, devoid of any entrapped air bubbles [7]. Various formulation batches were prepared according to the table. Plant extracts were excluded to make the gel base. The prepared gel formulations and base were kept at room temperature for 24 hours.

2: Composition of Gel Formulation

Ingredients	Quantity taken in (%) for gel base	Quantity taken in (%) for Formulation A	Quantity taken in (%) for Formulation B	Quantity taken in (%) for Formulation C
Carbopol 934	1.0	1.0	1.0	1.0
Extract of Neem	-----	0.1 (g)	0.3 (g)	0.5 (g)
Tea Tree Oil	-----	0.1 (mL)	0.3 (mL)	0.5 (mL)
Coconut Oil	-----	0.1 (mL)	0.3 (mL)	0.5 (mL)
Propylene glycol 400	4.0	4.0	4.0	4.0
Ethanol	3.0	3.0	3.0	3.0
Methyl paraben	0.2	0.2	0.2	0.2
Propyl paraben	0.02	0.02	0.02	0.02
EDTA	0.03	0.03	0.03	0.03
Triethanolamine	1.2	1.2	1.2	1.2
Water	Q.S. 100	Q.S. 100	Q.S. upto 100	Q.S. 100

3: Determination of Physical Appearance

The physical appearance of polyherbal gel tests were made as per the standardized protocols and method was taken from Dixit et al., 2013 except spreadability [8].

3(a) Determination of pH

A homogenous aqueous formulation was prepared by 1 g of the gel in 10 mL distilled water, the pH of the gels was determined using a pre standardized digital pH meter.

3(b) Determination of Drug Content

1 g of the gel was taken in 10ml of the volumetric flask containing 5ml of Ethanol and diluted to 5ml with the same solvent. From the above solution, 1ml was further diluted with 10ml ethanol. The resultant solution was filtered through Whatman filter paper and absorbance of the solution was measured at 268 nm using UV visible spectroscopy.

3(c) Determination of Viscosity

Viscosities of gels were determined using Brookfield viscometer. Gels were tested for

their rheological characteristics at 25°C using Brookfield viscometer. The measurement was made over the whole range of speed settings from 10rpm to 100rpm for 30seconds between 2 successive speeds and then in descending order.

3(d) Determination of Spreadability

Spreadability denotes the extent of the area to which the gel readily spread on application to the skin or the affected part. The bioavailability efficiency of a gel formulation also depends on its spreading value. The spreadability is expressed in terms of time in seconds taken by two slides slip off from the gel, placed in between the slides, under a certain load. The lesser time is taken for the separation of two slides, the better the spreadability. Two sets of glass slides of standard dimensions were taken. The herbal gel formulation (about 1 g) 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 6 cm along with the slide. A 500-gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 6cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated three times both formulated gels and marketed gel and the meantime is taken for the calculation [9].

Formula: $S=M \times L/T$

S=Spreadability, M= Mass in gm (30gm),
L=Length of the glass (6cm)

3(e) Determination of Extrudability

The formulated gel was filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weight of filled tubes was recorded and the tubes were sandwiched between two glass slides and were clamped. 500gm weight was placed over the slides and then the cap was removed to extrude. The amount of extruded gel was collected and weighed. Extrudability was determined by calculating the percentage of extruded gel [8].

When it is greater than 90% then extrudability is excellent.

When it is greater than 80% then extrudability is good.

When it is 70% then extrudability is fair.

4: Anti-bacterial activity

In-vitro antibacterial activity was evaluated using the agar well diffusion technique. The sterile agar plates were inoculated with the bacteria culture *Staphylococcus aureus* (MTCC 737) and *Pseudomonas aeruginosa* (MTCC 424) for 1 hour, at 37°C. Wells were bored by using a sterile borer, and standard gel formulations (1000 µg / ml was prepared by dissolving the test gel samples in DMSO) were placed into them. Next, the plates were incubated overnight (24 hours) at 37°C. The spectrum of activities of the gel formulation (F1, F2, and F3). After the incubation period, the diameter of the growth inhibition zones was measured [10].

Relative percentage inhibition of the test

$$\text{extract (r)} = \frac{100 (X-Y)}{(Z-Y)}$$

Where,

X= total area of inhibition of the test extract

Y= total area of inhibition of the solvent

Z = total area of inhibition of standard drug

r = radius of zone inhibition

RESULTS

Ethanol extract of Neem leaves, Tea Tree Oil, and Coconut oil, was selected to prepare polyherbal gel. This gel has been further analyzed for its physicochemical evaluation and antibacterial activity. Results of the present investigation were summarized in **Table 1, 2, 3 and 4**, revealed that this polyherbal gel has a significant spreadability,

extrudability, viscosity, and antibacterial activity value.

Physical properties

All three gel formulations were tested for physical parameter; these are experienced by human sense organs including finger touch (consistency), nasal smell, color and homogeneity etc. All results were summarized in **Table 1**. Physical properties are critical to understand or determine if the food or pharmaceutical products can transfer tastes or odors to the materials and components they are packed.

Melting point: The melting point of the extract was determined using the melting point apparatus. The melting point of the extract was found to be 118.

Drug Content of Polyherbal Gel

Drug content is the drug concentration of Gel, which was measured by a UV spectrophotometer. The drug content percentage of the gel was found to be within the range of 88.02% to 93.70%.

PHYSIOCHEMICAL PROPERTIES

The extrusion of the polyherbal gel from the tube is important during its application and in-patient compliance. Polyherbal gel with high consistency may not extrude from the tube whereas, the low viscous gel may flow quickly, and hence suitable consistency is required to extrude the polyherbal gel from the tube. The conventionally thickened polyherbal gel has good extrudability. The

extrudability, spreadability and viscosity of formulations given below in the **Table 2**.

Anti-bacterial activity

The antibacterial activity was evaluated by using the Well diffusion method. The bacterias used were *Pseudomonas aeruginosa*, *Staphylococcus aureus*. These bacteria are the major cause of most human skin infections. The results obtained from the antimicrobial assay were highly promising against *Staphylococcus aureus* while no results were found against *Pseudomonas aeruginosa*. The studies were carried for the best formulation and relative percentage inhibition of different gel formulations was observed as 22%, 33%, and 55% for the. F1, F2 and F3 respectively against *Staphylococcus aureus*. The results were satisfactory and showed that formulation 3 has the most promising antibacterial activity (**Table 3**).

In well diffusion assay, formulation F3 showed comparatively good antibacterial activity as compared to formulation F1 and F2.

Relative percentage inhibition of different gel formulations:

The relative percentage inhibition of gel formulations containing Neem, Tea tree oil, and Coconut oil for *Staphylococcus aureus* was found to be 22%, 33%, and 55%

respectively. From the study, F3 formulation was considered as the best anti-bacterial formulation since it shows maximum inhibition (Table 4).

Table 1: Physical parameters of poly-herb gel formulation:

Parameters Polyherb Preparation	Colour	Consistency	Odor	Taste
	Brown	Sticky paste-like	Characteristics	Bitter

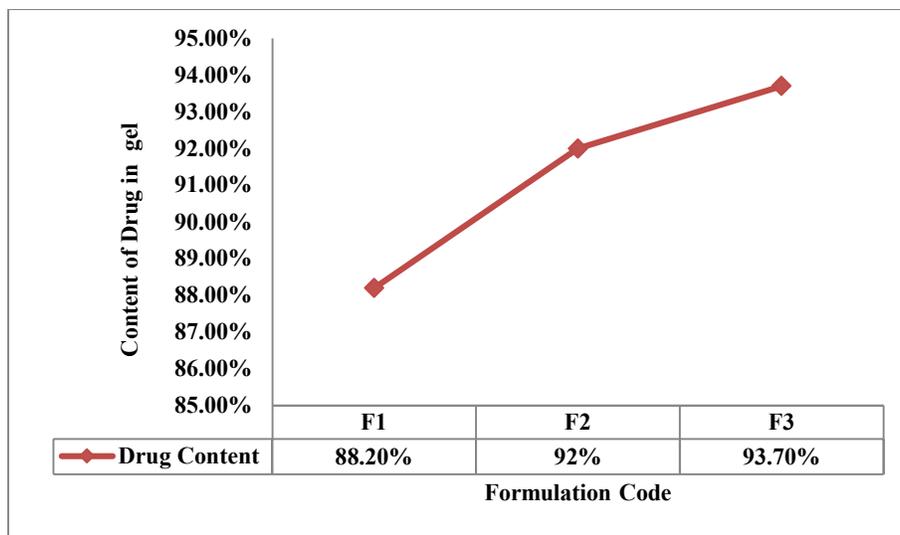


Figure 1: Graphical Representation of % Drug content of Polyherbal gel

Table 2: Physiochemical evaluation of polyherbal gel

Physiochemical Properties Formulation Code	Extrudability (g/cm ²)	Spreadability (cm)	Viscosity	
			Spindle speed (rpm)	cP
F1	9.3	3.48	20	31200
F2	8.9	4.32	20	31126
F3	10.3	3.90	20	31265

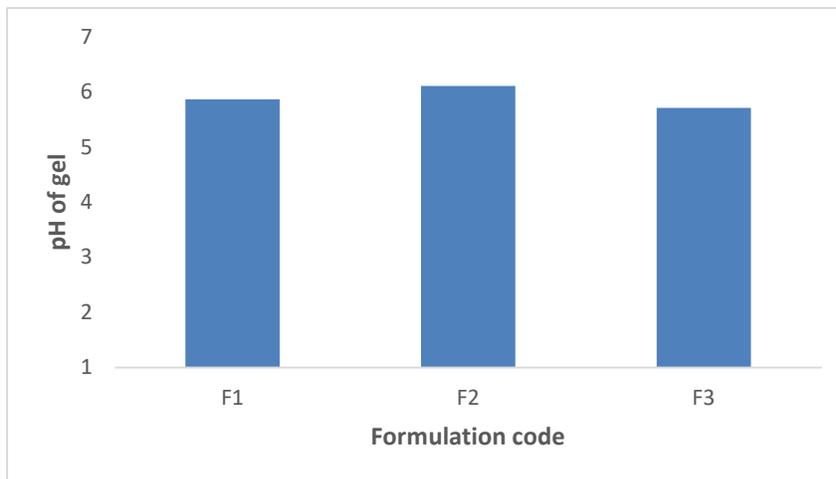


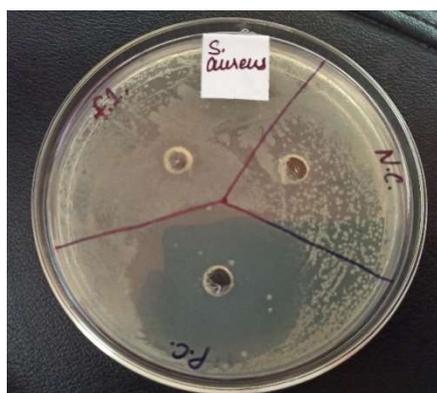
Figure 2: pH value of Polyherbal gel

Table 3: Antibacterial activity by Well diffusion method (-ve: no result; +ve good antibacterial activity)

Name of organism and code	Zone of Inhibition (mm) Well diffusion method		
	F1	F2	F3
<i>Pseudomonas aeruginosa</i> (MTCC 424)	-ve	-ve	-ve
<i>Staphylococcus aureus</i> (MTCC 737)	+ve	+ve	+ve

Table 4: Relative percentage inhibition of different gel formulations:

Test organism	Inhibition zone diameter (mm)		
	F1	F2	F3
<i>Staphylococcus aureus</i> (MTCC 737)	22%	33%	55%



A



B



C



D

Figure 3: (A), (B) and (C) positive control v/s DMSO v/s formulation F1, F2, and F3 respectively in well diffusion assay; (D) Evaluation of different concentrations (5 μ L, 10 μ L, and 20 μ L) of F3 in well diffusion assay

DISCUSSION:

An evaluation of all the prepared gel was done. The formulations were evaluated for different parameters such as homogeneity, pH, viscosity, spreadability, drug content, and extrudability. The visual inspection of all the prepared gel formulations showed good appearance and homogeneity. The pH value for the optimized gel. F1, F2, and F3 formulation was recorded as 5.87 to 6.11 respectively which is within the range of pH of the skin and would not cause any irritation to the skin. Thus, prepared gel formulations are suitable for skin application.

The extrudability of the best formulation F3 was found to be 10.3g/cm². Thus, the prepared gel possesses optimum extrudability. Viscosity reveals the rheological properties of all formulation. It should be optimized not too high or not too low according to topical applications.

The spreadability of gel was measured based on 'Slip' and 'drag' characteristics of gels. Spreadability is an important property of topical formulation from a patient compliance point of view. The formulations were evaluated for different parameters such as homogeneity, pH, viscosity, spreadability, drug content, and extrudability. The visual inspection of all the prepared gel formulations showed good appearance and

homogeneity. The pH value for the optimized gel is between. F1, F2, and F3 formulation was recorded as 5.87 to 6.11 respectively which is within the range of pH of the skin and would not cause any irritation to the skin. Thus, prepared gel formulations are suitable for skin application. The viscosity was found to be 31200cp, 31126cp, and 31265cp for F1, F2 and F3 respectively. The spreadability was found to be 3.48cm, 4.32cm, and 3.90cm for F1, F2 and F3 respectively. The drug content was found to be 88.2%, 92%, and 93.70, for F1, F2, and F3 respectively. Extrudability was found to be 9.3, 8.9, 10.3 for the formulations. F1, F2 and F3 respectively. Therefore, formulation F3 was found to be the best among three developed formulations, with better result as compare to others.

CONCLUSION

Thus, we can conclude that the formulated polyherbal gel containing Neem extract, Tea tree oil, Coconut oil can be used in the treatment of skin infections caused by *Staphylococcus aureus* such as impetigo, cellulitis, and folliculitis. Further, industrial manufacturing of this product can be taken up after conducting clinical trials on human volunteers.

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