Formulation And Evaluation of the Herbal Gel

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***Abstract*—There are various synthetic creams, gels, or other formulations currently available for the inflammation, but these have various side effects; hence, the study includes the herbal gel for the inflammation using Clerodendrum Serratum as the main ingredient, with other active ingredients and excipients The herbal gel is evaluated for the physical appearance, pH, viscosity, Spreadability, homogeneity, anti-microbial activity and the protein denaturation assay for checking the anti-inflammatory activity and the result shows the decrease in the absorbance as the concentration increases from 31.42% to 57.28% this result confirms the anti-inflammatory activity and anti-microbial activity hence the herbal gel is considered as safe and effective.**

***Index Terms*—HERBAL GEL, ANTI-INFLAMMATORY ACTIVITY, CLERODENDRUM SERRATUM, INDIAN SARSAPARILLA, ALSTONIA SCHOLARIS, AEGLE MARMELOS, WRIGHTIA TINCTORIA, NEEM POWDER, PROTEIN DENATURATION ACTIVITY**

I. INTRODUCTION

People are getting more interested in Herbal formulations these days. This is because they are worried about the effects of synthetic drugs and cosmetics. For a time, people have used plants with medicinal properties in traditional systems of medicine like Ayurveda, Siddha, and Unani to treat skin problems, wounds, and infections. The skin is an important part of our body, and it protects us from bad things like germs, pollution, and harmful radiation.

We have a lot of skin problems like acne, eczema, and fungal infections that need to be treated with effective medicines that we put on our skin. The problem is that many of these medicines have synthetic chemicals that can cause irritation, allergic reactions, or dryness after using them for a long time. So people are looking for topical formulations that are safe, do not cost a lot, and are good for our skin.

Indian Sarsaparilla is a plant that is used to reduce inflammation, purify blood, and kill germs [3,12]. Alstonia scholaris is another plant that can kill germs and help wounds heal [2,11]. Aegle marmelos is a plant that has antibacterial effects [6,13]. Clerodendrum serratum is used to reduce inflammation and pain [1,4,9]. Wrightia tinctoria is a plant that is used to treat skin diseases like psoriasis and eczema [5].

The goal of this research is to make a stable and effective herbal gel using extracts from these plants. We will check the gels appearance, pH level, thickness how well it spreads if it is uniform if it is stable and if it can kill germs. This study can help us make natural and effective herbal medicines that work well and do not have many bad effects. Herbal formulations like these can be very good, for our skin and overall health.

The present research aims to formulate, prepare, and evaluate a stable and effective herbal gel using selected medicinal plant extracts. The evaluation parameters include physical appearance, pH, viscosity, spreadability, homogeneity, stability, and antimicrobial activity. The study may contribute to the development of safe, natural, and effective herbal topical formulations with improved therapeutic efficacy and minimal side effects.

Figure 1 Crude plant materials

II. MATERIALS

The materials used for the herbal gel formulation are as follows: The herbal powders were collected from the Manakarnika Aushadhalaya, Chinchawad, Pune and authenticated at PES Modern College of Pharmacy, Pune. Indian Sarsaparilla root powder, Alstonia Scholaris leaf and bark powder, Clerodendrum Serratum root powder, Aegle Marmelos fruit powder, Wrightia Tinctoria root powder, Neem powder.

Chemicals:

Carbopol 934, Tri-ethanolamine, Ascorbic acid, Eucalyptus oil, Sorbitol.

III. METHOD

Step 1: Preparation of herbal extract by maceration
Weigh about 500 mg of each herbal powder. Transfer into a clean conical flask . Add sufficient amount of distilled water. Close the flask with an aluminum foil/cotton plug. Keep for 24–48 hours with occasional shaking. Filter using muslin cloth or filter paper. Collect the filtrate. Concentrate gently on hot plate required.

Step 2: Preparation of Gel Base
Take about 20 ml purified water in a clean beaker. Sprinkle Carbopol 934 (400 mg) slowly with continuous stirring to avoid lump formation. Use magnetic stirrer. Allow it to hydrate for 24 hours (or keep undisturbed for complete swelling). A clear, smooth viscous gel base will form.

Step 3: Addition of preservative
Prepared neem aqueous extract by taking 100 mg neem powder in 10 ml distilled water and heat 70-degree cs for 20 min. collect the filtrate. Add ascorbic acid into solution. add this solution into Carbopol base.

Step 4: Incorporation of herbal extract
Slowly add the prepared macerated extract to the Carbopol base. Stir continuously using glass rod or a magnetic stirrer. Mix until uniform.

Step 5: Neutralization and gel formation
Add triethanolamine dropwise. The stir gel continuously starts becoming thick and transparent. Adjust pH between 5.5 - 6.5

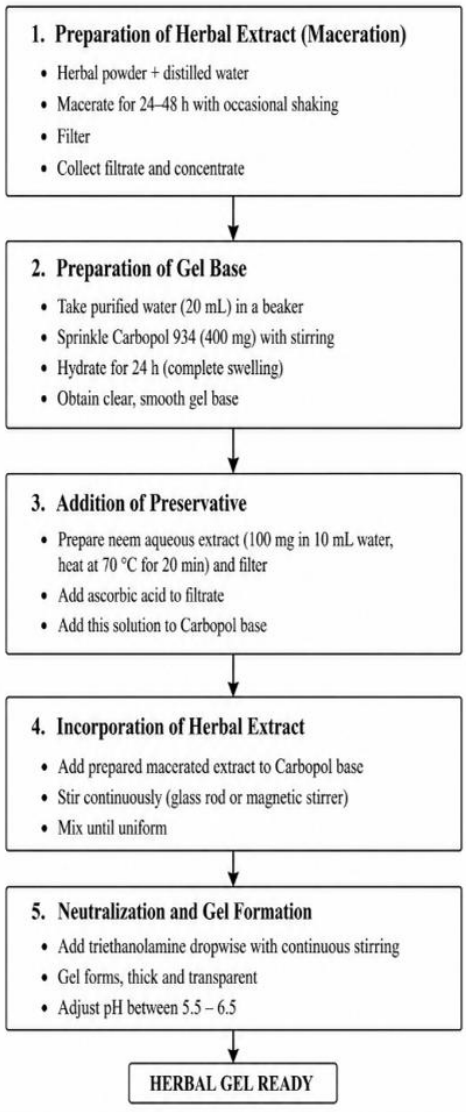

Figure 2 Flow chart for preparation of herbal gel

Ingredients	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Gelling agent	Carbopol 934 (300 mg)	Carbopol 934 (400 mg)	Carbopol 934 (400 mg)	Carbopol 934 (300mg)	Carbopol 934 (400 mg)
Indian sarsaparilla	1 ml	1 ml	1 ml	1 ml	1 ml
Alstonia scholaris	1 ml	1 ml	1 ml	1 ml	1 ml
Clerodendrum serratum	2 ml	2 ml	2 ml	2 ml	2 ml
Wrightia tinctoria	1 ml	1 ml	1 ml	1 ml	1 ml
Aegle marmelos	1 ml	1 ml	1 ml	1 ml	1 ml
Humectant	Sorbitol (2 ml)	Sorbitol (2 ml)	Sorbitol (2 ml)	Sorbitol (2 ml)	Sorbitol (2 ml)
Preservatives	Neem powder	Neem powder	Neem powder	Neem aqueos extract	Neem aqueous extract
Ascorbic acid	-	-	30 mg	30 mg	30 mg
Eucalyptus oil	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Triethanolamine	-	-	q.s	q.s	q.s
Distilled water	q.s	q.s	q.s	q.s	q.s

Figure 3 Flow chart for preparation of herbal gel

Table 1: Composition of the gel

Evaluations: [7,14,15]

1. Physical Appearance

The formulation is visually checked for its organoleptic properties. This involves assessing parameters such as the general appearance, colour, clarity, and texture of the prepared herbal gel and documenting the precise observations.

2. pH Determination

The pH value of the gel is measured using a calibrated digital pH meter. The sample solution is prepared by mixing a specific quantity of the herbal gel with distilled water to form a uniform suspension. This mixture is vigorously shaken before taking readings to ensure the formulation remains within acceptable skin-compatible physiological limits.

3. Viscosity

Transfer the herbal gel into a clean beaker without trapping air bubbles. Switch on the Brookfield viscometer. Select spindle no. 64 and 20 RPM rotational speed. Immerse the spindle in the gel sample up to the marked level. Allow the spindle to rotate for a few minutes until the reading becomes stable. Record the viscosity reading displayed on the instrument. Perform the test at room temperature (25 ± 2°C).

4. Homogeneity

The anti-inflammatory gel must demonstrate a completely uniform mass, texture, and distribution of active constituents. It is tested visually and texturally to confirm that it contains no aggregate lumps and extrudes as a continuous, consistent ribbon from a collapsible tube under normal pressure, without syneresis or separation of liquid phases.

5. Spradability test by using the tilt method: -

Place a clean glass slide on a horizontal surface. Put a fixed quantity of herbal gel (about 1 g) at one end of the slide. Tilt the slide at an angle of approximately **45°**. Allow the gel to flow downward due to gravity.

Measure the distance traveled by the gel within a fixed time (e.g., 1 minute). Record the observations. Formula: - S=T/L, Where:*S*= Spreadability ,*L*= Distance traveled by the gel (cm),*T*= Time taken (sec or min).

6. Stability test:-
Fill the herbal gel into suitable airtight containers. Store the samples under different conditions, such as Room temperature (25 ± 2°C) and refrigerated conditions (4 ± 2°C). Keep the samples for a specified period. To check Physical appearance, Color, odor, pH, Viscosity, Homogeneity, and spreadability.

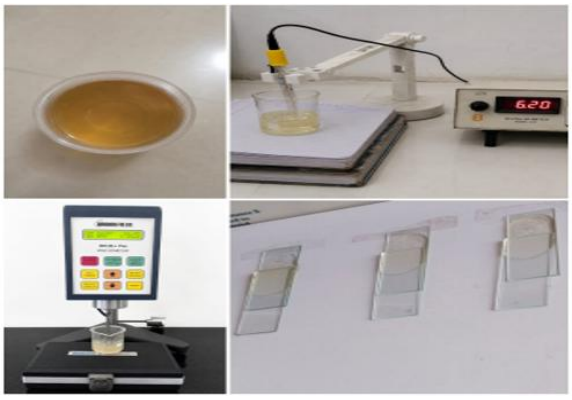

Figure 4 Evaluation of herbal gel

Table 2: Evaluation parameters of gel

Evaluations	Observations
Physical appearance	Slightly yellow
pH	6.20
Viscosity	16227 cP
Homogeneity	Good
Spradibility	0.6

7. Protein denaturation assay [8]:

Procedure
Prepare stock solutions
Stock A (NaH_2PO_4). Weigh 2.7 g NaH_2PO_4. Dissolve in distilled water. Make volume up to 100 mL
Stock B (Na_2HPO_4). Weigh 2.8 g Na_2HPO_4 Dissolve in distilled water. Make volume up to 100 mL

Prepare phosphate buffer
Using pipette or measuring cylinder :- Take 77 mL from NaH_2PO_4 stock. Take 23 mL from Na_2HPO_4

stock. Mix both in a clean beaker. Final volume = 100 mL phosphate buffer.

Egg Albumin
Weigh 100 mg of powdered egg albumin, pour it into a beaker add 10 ml of purified water.
Gently stir until fully dissolved. If necessary, adjust the pH.

Table 3: Test sample concentrations preparation:-

Concentration µg/ml	Stock (ml)	Buffer sol (ml)	Total volume(ml)
1000	1	9	10
2000	2	8	10
3000	3	7	10
4000	4	6	10

Standard preparation
Prepare 1mg/mL sample –weigh 100 mg of standard dissolved in 10 ml of buffer solution.

Activity procedure
The reaction mixture (5 mL) included 2.8 mL of phosphate buffered saline (PBS, pH 6.4), 0.2 mL of egg albumin and 2 mL of different concentrations of test sample, resulting in final concentrations of 1000, 2000, 3000, 4000 µg/mL.
As a control, a comparable volume of double-distilled water was used.
The mixtures were then incubated for 5 minutes at (37°C2) in a BOD incubator.
The mixtures were then heated to 70°C for 20 minutes
After cooling, their absorbance was measured at 277 nm using a vehicle as a blank.
In order to determine absorbance, standard drug was employed as a reference medication and treated similarly at final concentrations of 1000, 2000, 3000, 4000 µg/mL.

Calculations -

Inhibition of enzyme activity was calculated as (%) = (A-C)/ A X100, Inhibition of enzyme activity (%) = $\frac{A-C}{A} \times 100$

Observation: -

The herbal formulation exhibited significant anti-inflammatory activity by inhibiting protein denaturation in a concentration-dependent manner

Sr no	sample	Conc.µg/ml	Absorbance of sample(A)	Absorbance of control(C)	% inhibition of protein denaturation
1	Standard sample	1000	1.408	2.053	31.42
		2000	1.354	2.053	34.05
		3000	1.267	2.053	38.29
		4000	1.096	2.053	46.62
2	Test sample	1000	1.268	2.053	38.24
		2000	1.090	2.053	46.91
		3000	1.075	2.053	47.64
		4000	0.877	2.053	57.28

(Table 4)

Table 4: Protein denaturation assay

8. Antimicrobial activity

A swab of pure bacterial culture is evenly spread over Mueller-Hinton agar or nutrient agar plates. Using a cork borer, wells are punched in the agar. 100 µL of the samples is poured into the wells. The Petri plate is incubated for 18-24 hours at 37°C under optimal conditions for bacterial growth. After the incubation period, a clear area (zone of inhibition) around the antibacterial product sample is observed and measured

Observation

Less Active (Resistant): Zone size: < 10 mm. The drug is ineffective or has poor activity against the microorganism. The microorganism is resistant to the drug. Moderately Active (Intermediate): Zone size: 10–15 mm. The drug shows moderate activity, but it may not be sufficient to fully inhibit the microorganism under clinical

conditions. This suggests intermediate resistance. Good Activity (Susceptible): Zone size: > 15 mm. The drug is considered effective, with good antimicrobial activity against the microorganism. This indicates that the microorganism is susceptible to the drug, and it can be used to treat infections caused by this microorganism.

Figure 5 Test against Aureus and P. aeruginosa

Table 5: Zone of inhibition of Samples (Test) and standard against S.aureus

Sr no	Sample name	Zone of inhibition in mm	
1	control	NA	
1	Standard	23.65	
1		sample	20.49

Table 6: Zone of inhibition of Samples (Test) and standard against P.aeruginosa

Sr no	Sample name	Zone of inhibition in mm
1	control	NA
1	Standard	24.12
1	sample	22.35

IV. RESULT AND DISCUSSION

The protein denaturation assay revealed that the herbal gel formulation had significant anti-inflammatory properties, as it effectively reduced heat-induced protein denaturation. As the concentration of the sample increased, the level of inhibition also increased, showing that the activity was concentration-dependent. The standard sample inhibited protein denaturation by between 31.42% and 46.62% at concentrations ranging from 1000 to 4000 µg/ml, while the test sample showed a higher level of inhibition, ranging from 38.24% to 57.28% at the same concentrations. The highest inhibition was observed at 4000 µg/ml, where the test formulation outperformed the standard. This increased inhibitory effect is likely due to the presence of components such as flavonoids, tannins, and phenolic compounds in the herbal extracts, which help in stabilizing proteins and preventing their denaturation. These results suggest that the herbal gel has considerable anti-inflammatory potential and could be beneficial for managing inflammatory conditions when applied topically.
The antimicrobial study indicated that the herbal gel had strong antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa.

The test formulation produced inhibition zones of 20.49 mm and 22.35 mm against S.aureus and P.aeruginosa, respectively, while the standard sample produced inhibition zones of 23.65 mm and 24.12 mm. In contrast, the control sample showed no inhibition, indicating that the gel base itself did not contribute to antibacterial activity. The observed antimicrobial effect is attributed to the presence of phytoconstituents such as flavonoids, tannins, phenolic compounds, and terpenoids in the herbal extracts.

V. CONCLUSION

Based on the present investigation, the results demonstrated that the formulated gel exhibited significant anti-inflammatory and antimicrobial activities when compared with the standard formulation. The protein denaturation assay confirmed effective inhibition of protein denaturation in a concentration-dependent manner, indicating the promising anti-inflammatory potential of the herbal gel. The antimicrobial study also revealed appreciable activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, showing considerable zones of inhibition. The therapeutic activity of the formulation may be attributed to the presence of phytoconstituents such as flavonoids, tannins, phenolic compounds, alkaloids, and terpenoids present in the selected herbal extracts. These bioactive compounds are known to inhibit inflammatory mediators and suppress microbial growth. The herbal formulation showed enhanced activity due to the synergistic effect of combined herbal ingredients when compared to individual plant extracts. Therefore, the developed herbal gel may be considered a safe and effective topical preparation for the treatment of inflammation and microbial skin infections.

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