

DEVELOPMENT AND EVALUATION OF AN ARTEMISIA ANNUA HERBAL GEL FOR
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ABSTRACT

The present study was undertaken with the aim of developing and evaluating an *Artemisia annua* L. herbal gel for antimicrobial activity. The aerial parts of *A. annua* were collected from Bagsiad, Mandi, Himachal Pradesh, during September 2025 and authenticated by a qualified botanist. The collected plant material was shade-dried, powdered, and extracted with ethanol using the Soxhlet extraction method. The prepared extract was subjected to loss on drying and preliminary phytochemical screening to identify the major classes of secondary metabolites. The ethanolic extract was subsequently incorporated into Carbopol 940-based topical gel formulations. Five formulations (F1–F5) were prepared using different concentrations of Carbopol 940 and propylene glycol, while methyl paraben, propyl paraben, and triethanolamine were used as preservatives and pH-adjusting/neutralizing agents. The prepared formulations were evaluated for physical appearance, homogeneity, pH, viscosity, spreadability, and extrudability. Among the formulations, F2 was selected as the optimized formulation based on its overall physicochemical properties. F2 showed a smooth and homogeneous appearance, pH of 5.80 ± 0.046 , viscosity of 1285 ± 15.2 cP, spreadability of 6.80 ± 0.08 , and excellent extrudability. The optimized formulation was further evaluated for in-vitro antibacterial activity using the agar disc diffusion method against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. F2 exhibited zones of inhibition of 10 mm against *E. coli* and 11 mm against *P. aeruginosa*, whereas no zone of inhibition was observed against *S. aureus* under the experimental conditions. The findings indicate that the ethanolic extract of *A. annua* can be successfully incorporated into a Carbopol 940-based topical gel with acceptable physicochemical characteristics and measurable antibacterial activity. The developed F2 formulation may therefore have potential as a topical herbal antimicrobial preparation. Further studies are recommended to evaluate its stability, safety, skin irritation, drug release, permeation, and antimicrobial efficacy against a broader range of microorganisms.

KEYWORDS: *Artemisia annua*, Herbal gel, Carbopol 940, Ethanolic extract, Antimicrobial activity, Antibacterial activity, Topical formulation, Soxhlet extraction.**INTRODUCTION**

1. Medicinal plants have been used for centuries in traditional systems of medicine, including Ayurveda, Unani and Traditional Chinese Medicine, as important sources of therapeutic agents. They contain diverse primary and secondary metabolites responsible for various biological activities.^[1] Major phytochemical groups include alkaloids, flavonoids, phenolic compounds, terpenoids, tannins, glycosides, saponins, coumarins, steroids and essential oils. Among these, phenolic compounds

and flavonoids have received considerable attention because of their reported antioxidant, anti-inflammatory and antimicrobial properties.^[2] The phytochemical composition of medicinal plants may vary with species, geographical location, climatic conditions, soil characteristics, harvesting conditions and extraction methods. Therefore, proper authentication, processing and standardized extraction are essential for obtaining reproducible biological activity.^[3]

Antimicrobial agents are essential for preventing and treating infections caused by pathogenic microorganisms. However, the increasing emergence of antimicrobial resistance (AMR) has become a major global health concern. Resistant microorganisms can survive treatment and spread, leading to prolonged illness and increased therapeutic difficulties.^[4] Inappropriate antimicrobial use, unnecessary prescribing, poor treatment adherence and inadequate infection-control practices contribute to the development and dissemination of AMR.^[5] Consequently, medicinal plants are being investigated as potential sources of naturally derived antimicrobial compounds. Plant phytochemicals may inhibit microorganisms through

disruption of cell membranes, inhibition of enzymes and proteins, interference with cellular metabolism, alteration of microbial communication and inhibition of biofilm formation.^[6]

Artemisia annua L., commonly known as sweet wormwood or sweet Annie, belongs to the family Asteraceae. It is an aromatic annual herb containing several classes of biologically active phytoconstituents, including flavonoids, phenolic acids, terpenoids, coumarins, sesquiterpenes and essential oils. Previous investigations have reported antioxidant, anti-inflammatory and antimicrobial properties associated with *A. annua* extracts and essential oils.^[7]



Fig. 1: *Artemisia annua*.

Traditionally, *A. annua* has been used in Traditional Chinese Medicine and other traditional systems for conditions including fever and malaria. Various parts of the plant have also been traditionally associated with uses involving inflammation, pain, digestive disorders and other ailments. The presence of diverse secondary metabolites has contributed to continuing scientific interest in the pharmacological potential of the plant.^[8]

Topical administration is suitable for treating localized skin infections because it provides direct delivery of active substances to the affected site and may reduce unnecessary systemic exposure. Gels are widely used topical dosage forms because they are easy to apply, spread readily and can incorporate plant extracts effectively.^[9] Carbopol 940 is commonly used as a gelling agent because of its excellent thickening and gel-forming properties.^[10]

Therefore, the present study aimed to develop and evaluate an *Artemisia annua* herbal gel for antimicrobial activity. The study included ethanolic extraction, preliminary phytochemical screening, formulation of Carbopol 940-based herbal gels, evaluation of physicochemical properties, and *in-vitro* antibacterial assessment against selected microorganisms.

2. MATERIAL and METHODOLOGY

2.1 Collection and authentication of plant

Fresh aerial parts of *Artemisia annua* L. were collected from Bagsiad, Mandi, Himachal Pradesh, India, during September 2025. The plant material was authenticated by Dr. Ved Prakash, Assistant Professor of Botany, Government College Bassa, Gohar, Mandi, and Himachal Pradesh, India.

2.2 MATERIALS

Carbopol 940, propylene glycol, methyl paraben, propyl paraben and triethanolamine were obtained from standard commercial suppliers. Ethanol was used as the extraction solvent. Analytical-grade chloroform, ferric chloride, sodium hydroxide, hydrochloric acid, sulfuric acid, glacial acetic acid and distilled water were used for preliminary phytochemical screening.

The major equipment used during the study included a Soxhlet extraction apparatus, digital analytical balance, hot-air oven, digital pH meter and Brookfield viscometer.

2.3 Preparation of *Artemisia annua* Extract

The collected aerial parts of *A. annua* were cleaned and shade-dried under suitable conditions. The dried material was powdered and subjected to Soxhlet extraction using

ethanol. Extraction was carried out at approximately 60–70°C. The resulting extract was concentrated by removal

of the solvent and subsequently dried for further evaluation and formulation.^[11,12]



Fig. 2: Hot continuous extraction (Soxhlet).

2.4 Preliminary Phytochemical Screening

The ethanolic extract was subjected to qualitative phytochemical screening for flavonoids, glycosides, terpenoids, tannins, phenols and saponins using standard chemical tests, including alkaline reagent, lead acetate, Keller–Killiani, Legal's, Salkowski, ferric chloride, gelatin, Liebermann–Burchard and foam tests.^[13,14]

2.5 Formulation of *Artemisia annua* Herbal Gel

Herbal gels were prepared using the dispersion method with Carbopol 940 as the gelling agent. Carbopol 940 was dispersed in distilled water and allowed to hydrate for 24 h. The ethanolic extract was dissolved in ethanol and mixed with propylene glycol, followed by incorporation of methyl paraben and propyl paraben. The extract solution was gradually added to the hydrated Carbopol dispersion with continuous stirring. Triethanolamine was added dropwise to neutralize the Carbopol dispersion and obtain the desired gel consistency, while the final pH of each formulation was subsequently determined. Five formulations (F1–F5) were prepared by varying the concentrations of Carbopol 940 and other excipients while maintaining 1 g of *A. annua* extract in each formulation.^[15,16]

2.6 Evaluation of Herbal Gel

The prepared *Artemisia annua* herbal gel formulations were evaluated for physical appearance, pH, viscosity, spreadability, homogeneity, extrudability, and washability.

a. Physical Appearance

The formulations were visually examined for colour, transparency, consistency, smoothness, lumps, grittiness, air bubbles, and phase separation. A good gel should be smooth, uniform, and free from visible defects.

b. pH

One gram of gel was dispersed in 10 mL of distilled water, and the pH was measured using a calibrated digital pH meter. The pH was evaluated for suitability for topical application.

c. Viscosity

The viscosity of the formulations was measured using a Brookfield viscometer. The viscosity of the gel was assessed to determine its suitability for topical application and spreadability.

d. Spreadability

About 0.5 g of gel was placed between two glass slides, and a 100-g weight was applied for 5 minutes. The spreadability of the gel was then assessed by measuring the spread of the formulation.

e. Homogeneity

The formulations were examined visually for uniformity, lumps, aggregates, grittiness, and phase separation. The distribution of the extract throughout the gel was also assessed. A good formulation should have a smooth and uniform texture.

f. Extrudability

The gel was filled into collapsible tubes, and extrudability was evaluated by applying gentle pressure to the tube. The ease of extrusion of the gel was then observed.

g. Washability

A small amount of gel was applied to the skin and subsequently washed with water. The ease of removal and any visible residue remaining on the skin were observed.^[17,18]

2.7 In-vitro Antimicrobial Activity

The antimicrobial activity of the optimized herbal gel was evaluated by the agar disc diffusion method against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Standardized bacterial suspensions equivalent to 0.5 McFarland were uniformly

inoculated onto suitable agar plates. Discs containing the herbal gel were placed on the inoculated plates and incubated under appropriate conditions. A gel base without extract was used as the negative control, while a suitable standard antimicrobial agent served as the

positive control. After incubation, the zones of inhibition were measured in millimetres and used to assess the antibacterial activity of the formulation.^[19,20]

3. RESULTS AND DISCUSSION

Table 1: Preliminary phytochemical analysis.

Sr. no.	Phytochemicals	Phytochemical Test	Result
1.	Flavonoid	Lead Acetate Test	+
2.	Terpenoids	Salkowski Test	+
3.	Glycosides	Keller -Killiani Test	Weakly present
4.	Tannins	Ferric Chloride Test	-
5.	Phenolic compounds	Ferric Chloride Test	+
6.	Saponins	Foam Test	Weakly present

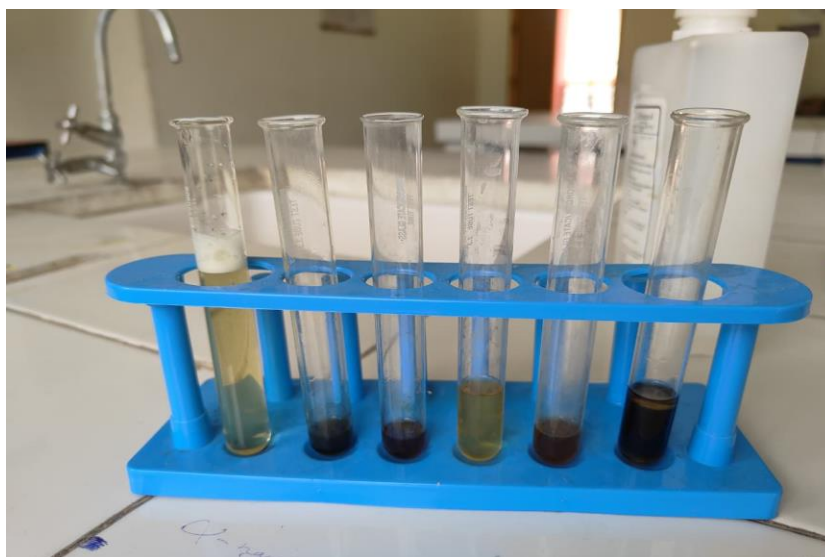


Fig. 3: Phytochemical Tests for *Artemisia annua* Extract.

Table 2: Composition of Herbal Gel.

Chemicals	F1	F2	F3	F4	F5
Artemisiaextract	1 g	1 g	1 g	1 g	1 g
Carbopol 940	0.75g	0.52g	1.00g	1.25g	1.50g
Propyleneglycol	4ml	5ml	5ml	6ml	7ml
Methyl paraben	0.10g	0.10g	0.10g	0.10g	0.10g
Propyl paraben	0.05g	0.04g	0.03g	0.07g	0.03g
Triethanolamine	Upto pH 6.8	Upto pH 6.8	Upto pH 6.8	Upto pH 6.8	Upto pH 6.8
Distilled water q.s.to 50ml	q.s.	q.s.	q.s.	q.s.	q.s.

3.3 Organoleptic properties of Herbal Gel Formulations

Table 3: Organoleptic Properties of *Artemisia annua* Aerial Parts.

Parameters	Observations
Touch/Texture	Slightly rough, soft leaves with firm stems
Colour	Green to brownish-green
Taste	Bitter
Odour	Characteristic aromatic

3.3 Evaluation of Herbal Gel Formulations

Five *A. annua* herbal gel formulations (F1–F5) were prepared using Carbopol 940 with varying polymer and propylene glycol concentrations. All formulations showed acceptable physical appearance without phase

separation. F2, F3 and F5 exhibited smooth and homogeneous texture, whereas slight lumps were observed in F1 and F4.

The pH of the formulations ranged from 5.60 ± 0.073 to 6.10 ± 0.091 , indicating suitability for topical application. Viscosity increased from 1245 ± 18.6 to 1450 ± 26.3 cP, while spreadability ranged from $5.50 \pm$

0.14 to 6.80 ± 0.08 . F2 showed the highest spreadability and excellent extrudability, making it more suitable for topical application.

Table 4: Appearance Evaluation of Herbal Gel Formulations.

Formulation	Colour	Appearance	Consistency	Phase separation
F1	Light green	Smooth	Slightly thin	Absent
F2	Light green	Smooth	Good	Absent
F3	Light green	Smooth	Uniform	Absent
F4	Light green	Smooth	Thick	Absent
F5	Dark green	Very thick	Highly viscous	Absent

Table 5: Evaluation of *Artemisia annua* Herbal Gel Formulations.

Parameter	F1	F2	F3	F4	F5
pH	5.60 ± 0.073	5.80 ± 0.046	5.70 ± 0.082	6.00 ± 0.067	6.10 ± 0.091
Viscosity (cP)	1245 ± 18.6	1285 ± 15.2	1320 ± 21.4	1385 ± 24.7	1450 ± 26.3
Spreadability	6.20 ± 0.12	6.80 ± 0.08	6.40 ± 0.15	5.90 ± 0.11	5.50 ± 0.14
Extrudability	Good	Excellent	Good	Poor	Moderate
Homogeneity	Slight lumps	Smooth	Homogeneous	Slight lumps	Smooth

Based on the combined physicochemical evaluation, F2 was selected as the optimized formulation because of its suitable pH, viscosity, highest spreadability, smooth homogeneity, and excellent extrudability.

3.4 Selection of Optimized Formulation

Based on the combined physicochemical characteristics, formulation F2 was selected as the optimized formulation. F2 exhibited a smooth appearance and good homogeneity, a pH of 5.80 ± 0.046 , a viscosity of 1285 ± 15.2 cP, the highest spreadability value of 6.80 ± 0.08 among the tested formulations, and excellent extrudability.

The selection of F2 was based on the overall balance of its physicochemical properties rather than on a single parameter. Its moderate viscosity, combined with

comparatively high spreadability and excellent extrudability, indicated favourable handling and application characteristics for topical administration. Therefore, F2 was selected for further evaluation of its antibacterial activity.

3.5 Antimicrobial Activity

The antimicrobial activity of optimized formulation F2 was evaluated by the agar disc diffusion method against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. F2 showed measurable antibacterial activity against *P. aeruginosa* and *E. coli*, producing zones of inhibition of 11 mm and 10 mm, respectively. No zone of inhibition was observed against *S. aureus*. The observed activity may be attributed to bioactive constituents of *A. annua*, including flavonoids, phenolic compounds and terpenoids.

Table 6: Antimicrobial Activity of Optimized Herbal Gel (F2).

S. No	Microorganisms	F2
1.	E.Coli	10mm
2.	S.Aureus	No Zone
3.	Pseudomonas	11mm

Overall, the antibacterial activity followed the order: *P. aeruginosa* (11 mm) > *E. coli* (10 mm) > *S. aureus* (no zone).



Fig. 4: E. coli.



Fig. 5: Staphylococcus Aureus.

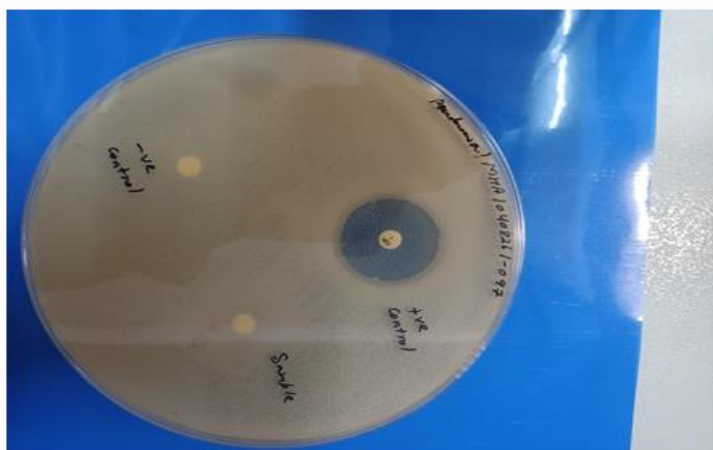


Fig. 6: Pseudomonas aeruginosa.

4. CONCLUSION

The present study was undertaken with the aim of developing and evaluating an *Artemisia annua* L. herbal

gel for antimicrobial activity. The aerial parts of *A. annua* were collected from Bagsiad, Mandi, Himachal Pradesh, authenticated, shade-dried, and subjected to

ethanolic extraction using the Soxhlet extraction method. The prepared extract showed a loss on drying of 6% and preliminary phytochemical screening indicated the presence of various bioactive phytoconstituents. Five herbal gel formulations (F1–F5) were prepared using Carbopol 940 as the gelling agent along with propylene glycol, methyl paraben, propyl paraben, and triethanolamine. The formulations were evaluated for appearance, homogeneity, pH, viscosity, spreadability, and extrudability. Among the prepared formulations, F2 was selected as the optimized formulation because it exhibited a smooth and homogeneous appearance, suitable pH (5.80 ± 0.046), desirable viscosity (1285 ± 15.2 cP), highest spreadability (6.80 ± 0.08), and excellent extrudability. The optimized formulation F2 was further evaluated for in-vitro antibacterial activity by the agar disc diffusion method and showed zones of inhibition of 11 mm against *Pseudomonas aeruginosa* and 10 mm against *Escherichia coli*, whereas no zone of inhibition was observed against *Staphylococcus aureus* under the experimental conditions. Overall, the findings demonstrate that ethanolic extract of *Artemisia annua* can be successfully incorporated into a Carbopol 940-based topical gel with acceptable physicochemical properties and measurable antibacterial activity, particularly against the tested Gram-negative bacteria. Therefore, the developed *A. annua* herbal gel, particularly formulation F2, may be considered a promising preliminary topical herbal formulation with antibacterial potential; however, further studies involving additional microbial strains, quantitative antimicrobial testing, stability, safety, skin irritation, drug-release, and permeation studies are recommended to establish its efficacy and suitability for future topical pharmaceutical application.

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