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FORMULATION AND EVALUATION OF SEMISYNTHETIC ANTIFUNGAL INSITU GEL CONTAINING SENNA AURICULATA AND AZADIRACHTA INDICA LEAVES

Gawade Sangram Santosh[1], Ingale Gauri Raosaheb[1], Pachpute Madhuri Rajendra[1], Gunjal Sujit Sakharam[1], Sake Vaishnavi Sanjay[2], Dr. Veer Sujata Umakant[2], Dr. Khedkar Amol Navnath[3]*

[1]Student, Shri Dattakrupa Foundations, Saikrupa Institute of Pharmacy, Ghargaon, Shrigonda, Ahilyanagar, Maharashtra, India 413728.

[2]Assistant Professor, Shri Dattakrupa Foundations, Saikrupa Institute of Pharmacy, Ghargaon, Shrigonda, Ahilyanagar, Maharashtra, India 413728.

[3]Principal, Shri Dattakrupa Foundations, Saikrupa Institute of Pharmacy, Ghargaon, Shrigonda, Ahilyanagar, Maharashtra, India 413728.

*** Corresponding author: Dr. Khedkar Amol Navnath[3]*,**
Email: dr.amol.n.khedkar@gmail.com

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KEYWORDS

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Abstract

The antimicrobial in-situ gel containing extracts of *Senna auriculata* flower and *Azadirachta indica* leaves for topical drug delivery. Herbal medicines are widely used for the treatment of skin infections and inflammatory conditions due to their therapeutic effectiveness, safety, easy availability.
The prepared formulations were evaluated for various physicochemical parameters including appearance, color, odor, homogeneity, pH, viscosity, spread ability, gelation capacity. Spread ability was determined to evaluate ease of application, while viscosity studies were performed using Brookfield viscometer to determine rheological behavior of the gel. The pH of the formulation was maintained within the range suitable for skin application. The antimicrobial activity of the prepared in-situ gel was evaluated by agar well diffusion method against selected microorganisms including Staphylococcus aureus, Escherichia coli, and Candida albicans. The optimized formulation exhibited significant antimicrobial activity with satisfactory zone of inhibition against test organisms. Stability studies were carried out under different storage conditions to evaluate physical stability, pH, viscosity, and homogeneity of the formulation. The results of the study demonstrated that the formulated in-situ gel showed satisfactory physicochemical characteristics, good spread ability, and acceptable viscosity, excellent and significant antimicrobial activity.

INTRODUCTION

Fungal skin infections are the most prevalent infectious diseases worldwide, affecting millions of individuals annually. Conditions such as dermatophytosis, candidiasis, and superficial mycoses cause significant discomfort and may lead to chronic infections. Conventional antifungal therapies are often associated with limitations including poor patient compliance, frequent dosing requirements, inadequate drug penetration, and the emergence of resistant fungal strains. These challenges have encouraged the exploration of novel topical drug delivery systems and plant-based therapeutic agents with improved efficacy and safety profiles. In-situ gel systems represent an advanced drug delivery approach that undergoes sol-to-gel transformation upon application, thereby increasing residence time at the site of administration and enhancing drug retention. [9] Such systems offer

advantages including ease of application, prolonged contact with infected tissues, controlled drug release, improved patient compliance, and enhanced therapeutic effectiveness..

The concept of a semisynthetic formulation combines the therapeutic benefits of natural herbal extracts with the stability, consistency, and performance characteristics of synthetic pharmaceutical excipients. This approach enables the development of a formulation possessing desirable physicochemical properties, adequate viscosity, appropriate pH, sustained drug release, and enhanced antifungal activity. Therefore, the present study was undertaken to formulate and evaluate a semisynthetic antifungal in-situ gel containing *Senna auriculata* flower extract and *Azadirachta indica* leaf extract. The formulated gel was evaluated for various physicochemical parameters including appearance, pH, viscosity, gelation characteristics, spreadability, drug content, stability, and antifungal activity to assess its potential as an effective topical antifungal drug delivery system. [4].

pH sensitive systems

The gelation behavior of pH responsive formulations is influenced by the constituent polymer's pKa. Weakly acidic or basic pH sensitive polymeric solutions typically transform into gel at pH levels lower than their pKa values or greater than their pKa, respectively. In addition, variation in the pH of the physiological environment could facilitate changes in the Polymer's ionization state, conformation, and solubility, resulting in the gelation of the drug carrier. Typically, pH sensitive polymers have ionizable weakly basic or weakly acidic moieties on their backbone. For example, polymers with weakly acidic groups, such as poly (methacrylic acid) containing carboxylic acid, become deprotonated at alkaline pH (above their pKa values) and acquire negative charge, resulting in increased electrostatic repulsion between polymer molecules which can in turn lead to physical transitions pH lower than the pKa can thus promote polymer–polymer interactions, such as H-bonding between COOH pairs, which are required for gel formation. pH above the pKa will transition the polymer to a polyelectrolyte which is likely to lead to polymer–polymer repulsion and will typically form viscous solutions, dependent upon factors including molecular weight and ionic strength of solution [10].

Mechanism of in-situ gel

The mechanism of in-situ gel involves transformation of liquid formulation into gel under physiological conditions. In Carbopol-based systems, pH change causes polymer expansion and formation of a three-dimensional gel network.

This mechanism improves retention time, sustained drug release, and therapeutic efficacy of herbal formulations.

The gel formation in Carbopol-based in-situ gel occurs mainly due to pH change. Carbopol polymer remains in liquid state at acidic pH but converts into gel after neutralization with triethanolamine. [8]

Advantages of In-Situ Gel

1. Prolonged residence time at application site
2. Sustained and controlled drug release
3. Improved bioavailability
4. Easy administration
5. Reduced frequency of application
6. Better patient compliance
7. Enhanced stability of formulation
8. Non-greasy and washable nature

MATERIALS AND METHODS

Materials

***Senna Auriculata* Flower**

Figure 1: *Senna Auriculata* Flower

Senna auriculata, commonly known as Tanner's Cassia or Avaram, belongs to the family Fabaceae. It is a traditional medicinal plant widely distributed in India and other tropical regions. Different parts of the plant such as flowers, leaves, bark, roots, and seeds are used in traditional systems of medicine including Ayurveda and Siddha. Among these, the flowers are especially valued because of their rich phytochemical composition and therapeutic potential The flowers of *Senna auriculata* contain various bioactive constituents such as flavonoids, tannins, phenolic compounds, glycosides, saponins, anthraquinones, and terpenoids. These phytoconstituents are responsible for important medicinal properties including antimicrobial, antioxidant, anti-inflammatory and wound healing activities. Due to these properties, the flower extract is considered highly suitable for topical pharmaceutical formulations.

Neem Leaves:

Figure 2: Neem Leaves

Neem belongs to the family Meliaceae and is commonly known as Neem or *Indian Lilac*. It is widely distributed throughout India and other tropical countries. Different parts of the plant such as leaves, bark, seeds, flowers, and fruits are used traditionally for the treatment of various diseases. Among these, neem leaves possess remarkable medicinal properties and are extensively used in herbal pharmaceutical preparations. The leaves of Neem contain several important phytoconstituents such as flavonoids, tannins, alkaloids, glycosides, saponins, terpenoids, phenolic compounds, and limonoids including azadirachtin and nimbin. These bioactive compounds are responsible for various pharmacological activities including antimicrobial, antifungal, antioxidant, anti-inflammatory, wound healing, and antiseptic properties. Microbial infections affecting skin and wounds are common health concerns requiring effective treatment.

Table 1: Ingredient and their role

Sr. No.	Ingredient	Role
1.	Senna auriculata extract	Antimicrobial
2.	Neem leaves extract	Antibacterial
3.	Carbopol 934	Gelling agent
4.	HPMC	Viscosity enhancer
5.	Propylene glycol	Penetration enhancer
6.	Methyl paraben	Preservative
7.	Propyl paraben	Preservative
8.	Triethanolamine	Neutralizing agent
9.	Distilled water	Solvent

Method:

A. Collection of Plant Material

The fresh plant materials of *Senna Auriculata* flower and Neem leaves were collected from local area (Kashti, Shrigonda, Ahmednagar dist.) And authenticated at Department of Botany, Radhabai Kale Mahila Mahavidyalaya Ahilyanagar.

B. Preparation of powder

Remove dust, dirt, and unwanted materials. Wash flowers and leaves thoroughly with distilled water. Removes contaminants and impurities. Spread flowers and leaves on a clean tray. Dry under shade at room temperature for 7-8 days. Avoid direct sunlight because it may destroy active constituents. Drying continues until flowers become Moisture free dried flowers are crushed using grinder, convert into coarse powder, and then pass powder through sieve. Fine powder is collected.

C. Preparation of extraction

- Take a 40 gm of powder of both drug in beaker and was soaked in 400 ml ethanol.
- Take a side for 5-7 days covered with aluminum foil.
- Shake the extract two times a day.
- The mixture was filtered through the filter paper (Whatman No.1).
- Stored in sterile amber bottles at 4 ℃ until further use.

D. Phytochemical screening

1) **Dragendorff's Test**

Procedure: Take 2 ml of plant extract. Add few drops of Dragendorff's reagent.

Observation: Orange color appears.

Inference: Presence of alkaloid

2) Shinoda Test

Procedure: Take 2 ml extract.Add small magnesium turnings. Add few drops of concentrated HCL

Observation: Red color develops.

Inference: Presence of flavonoids

3) Ferric Chloride Test

Procedure**:** Take 2 ml extract**.** Add few drops of 5% ferric chloride solution.

Observation**:** Greenish-black color appears.

Inference**:** Presence of tannins.

4) Foam Test

Procedure: Shake 2 ml extract with 5 ml distilled water vigorously.

Observation: Stable persistent foam appears for 10–15 minutes.

Inference: Saponins present.

5) Keller–Killiani Test

Procedure: Take 2 ml extract. Add glacial acetic acid. Add ferric chloride.Carefully add concentrated sulfuric acid.

Observation: Brown ring appears at junction.

Inference: Glycosides present.

6) Salkowski Test

Procedure: Take 2 ml extract. Add chloroform. Add concentrated sulfuric acid carefully.

Observation: Reddish-brown color appears.

Inference: Terpenoids present.

Figure 3: Phytochemical Test

Preperation of In-situ Gel

Step 1: Preparation of Polymer Solution

Step 2: Preparation of Preservative Solution

Step 3: Incorporation of Plant Extracts

Step 4: Mixing of Components

Step 5: pH Adjustment and Gel Formation

Step 6: Final Volume Adjustment

Step 7: Removal of Air Bubble

Formulation of In-situ Gel

Table 2: Formulation Table

Sr. no.	**Ingredient**	**Batch F1 10 gm**	**Batch F2 10 gm**	**Batch F3 10 gm**	**Batch F4 10 gm**	**Batch F5 10 gm**
1	Senna auriculata extract	0.5	0.5	0.5	**0.5**	0.5
2	Neem leaves extract	0.5	0.5	0.5	**0.5**	0.5
3	Carbopol 934	1	0.8	0.6	**0.3**	0.4

4	HPMC	0.8	0.5	0.2	**0.15**	0.18
5	Propylene glycol	2.3	1.8	1.5	**1**	1.3
6	Methyl paraben	0.08	0.06	0.05	**0.02**	0.04
7	Propyl paraben	0.04	0.03	0.02	**0.01**	0.02
8	Triethanolamine	q.s	q.s	q.s	**q.s**	q.s
9	Distilled water	q.s to 10	q.s to 10	q.s to 10	**q.s to 10**	q.s to 10

Evaluation Parameter

1. Physical Appearance

To check colour odour appearance of gel

2. Stability Test

These test is not carried out at any fixed temperature. In this test, temperature was changed every day. At room temperature and frizzing temperature to stimulates the change temperature

3. Determination of pH:

The pH of the prepared formulation was determined by using digital pH meter by preparing 10% solution and dipping the glass electrode completely in the solution system to cover the electrode.

4. Viscosity:

Viscosity of insitu gel was determined using brook filled viscometer at 25 ^{0}C with a spindle speed of the viscometer rotated that 12rpm.

5. Spreadability:

The spreadability was determined by placing sample between two glass slides which was compressed to uniform thickness by applying definite time period. The time required to separate the two slides was measured as spreadability less time taken for separation of two slides shown better spreadability calculated by formula S=M*L/T

6. Homogeneity Test

To check uniformity of formulation.

Procedure: Visually inspect gel after preparation. Check for lumps and phase separation.

Rub gel between fingers to examine texture.

7. Gelation Study

To determine gel forming capacity.

Procedure: Take small quantity of formulation in test tube. Add simulated physiological fluid.

RESULT AND DISCUSSION

A. Physical Evaluation

Table 3: Physical evaluation

Formulation	Color	Consistency	Odour
F1	Dark Brownish	Moderate	Characteristic
F2	Dark Brownish	Poor	Characteristic
F3	Light Brownish	Moderate	Characteristic
F4	**Light Brownish**	**Good**	**Characteristic**
F5	Light Brownish	Good	Characteristic

B. Stability study

Table 4: Stability Study

Formulation	**Room temperature**	**Freezing Temperature**
F1	Unstable	Unstable
F2	Unstable	Unstable
F3	Stable	Unstable
F4	**Stable**	**Stable**
F5	Stable	Stable

C. Measurement of ph.

Table 5: Measurement of pH

Formulation	**pH**
F1	6.8
F2	6.6
F3	6
F4	**5.8**
F5	6.2

D. Gelation study

Table 6: Gelation study

Formulation	**Gelation time**
F1	2 min
F2	1 min 30 sec
F3	1 min
F4	**45 sec**
F5	30 sec

E. Determination of Zone Inhibition

Antifungal study Method- Zone of Inhibition

Materials and methods:

Method: Zone of Inhibition (Agar Well Diffusion Method)

Media: Sabouraud Dextrose Agar (SDA)

Culture Used

Fungal Culture Used: *Candida albicans , Aspergillus niger*

Incubation Temperature: 28 ± 2°C

Incubation Time: 24–48 Hrs

Standard Drug: Fluconazole

Results and conclusion:

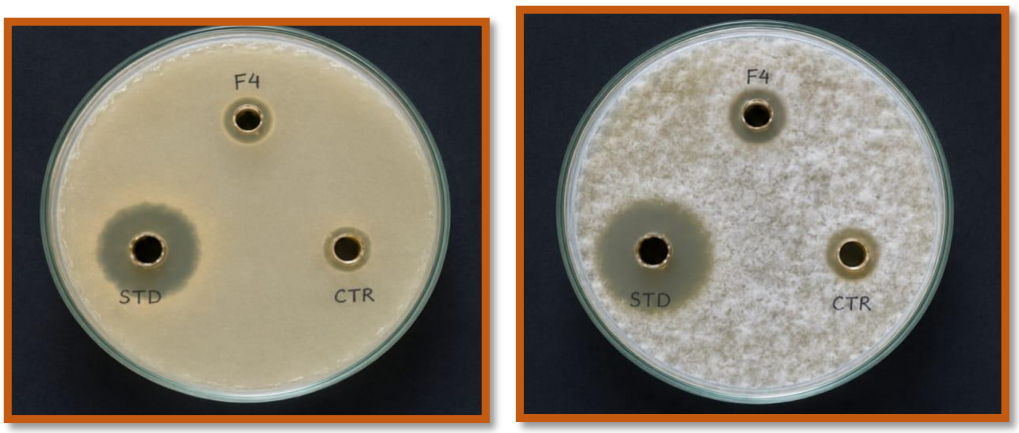
Sample F4 showed the highest antifungal activity among all formulations.

The antifungal activity of F4 was comparable to the standard drug Fluconazole.

The semisynthetic in-situ gel containing *Senna auriculata* flower extract and neem leaf extract demonstrated significant antifungal potential and may be useful for the treatment of fungal skin infections.

Table 7: Zone of Inhibition

Sample ID	Conc. of Stock solution	Zone of inhibition in mm			
		Candida albicans **NCIM 3471**	***Aspergillus niger*** **NCIM 501**	***Trichophyton Rubrum*** **NCIM 2816**	***Microsporum canis*** **NCIM 5775**
F4	*Senna Auriculata* flower and Neem Leaves Insitu gel (Formulation 10 µg/ml)	24 ± 0.35	23 ± 0.32	22 ±0.30	21 ± 0.27
Standard Solution	Flucanazole (0.5 mg/ml)	26 ± 0.28	24 ± 0.25	23 ± 0.24	22 ± 0.23
Control Solution	Blank gel	0	0	0	0



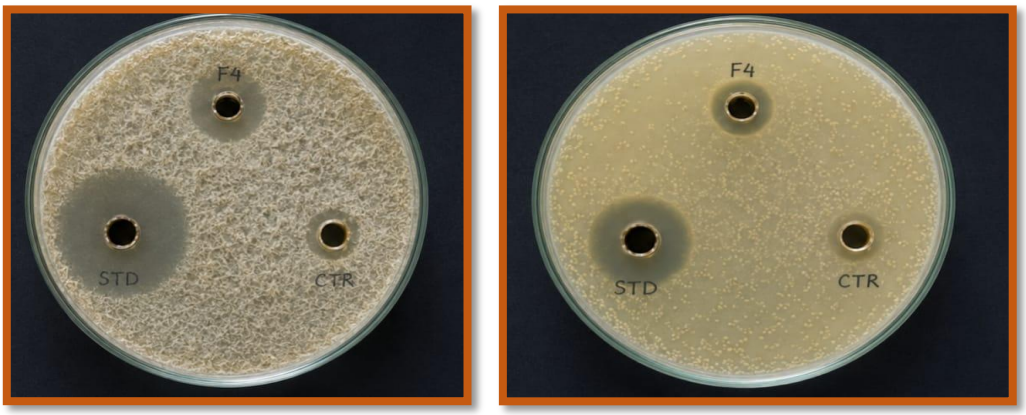


Figure 3: Zone of Inhibition

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SUMMARY AND CONCLUSION

Summary

In-situ gels has received extensive attention over the past few years as they are capable of undergoing rapid solution-to-gel transformation due to physico-chemical changes occurring in the eye. Hence the developed sulfaceacetamide sodium in situ gel formulation is a better alternate to exisiting conventional eye drops by virtue of improved precoreneal residence time of dosage form at the site of administration, sustained action, better therapeutic efficiency and consequently reduces the dosing frequency with improved patient compli-ance and convenience

Conclusion

The present study successfully formulated and evaluated an antimicrobial in-situ gel containing extracts of *Senna auriculata* flower and Neem leaves using Carbopol 934 and HPMC as gelling polymers. The developed formulation demonstrated satisfactory physicochemical characteristics including acceptable pH, good homogeneity, suitable viscosity, rapid gelation capacity, and adequate spreadability, which are essential properties for topical in-situ gel systems.

The optimized formulation exhibited pseudo plastic flow behavior and rapid sol-to-gel transition, indicating effective retention and sustained release potential at the site of application. Antimicrobial evaluation confirmed significant inhibitory activity against selected microorganisms due to the synergistic action of bioactive phytoconstituents present in *Senna auriculata* and Neem extracts.

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