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Formulation and Evaluation of Mucoadhesive Gel of Eugenol for Antifungal Activity

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ABSTRACT
The present study aimed to formulate and evaluate a mucoadhesive vaginal gel containing eugenol as the principal active agent for the treatment of vaginal fungal infections. Eugenol, known for its broad-spectrum antimicrobial activity, was incorporated into a gel base using Carbopol 934, with methyl paraben as a preservative and phosphate buffer as the vehicle. Preformulation studies including organoleptic evaluation, boiling point determination, solubility analysis, pH measurement, and UV spectroscopic analysis confirmed the identity and stability of eugenol. Phytochemical screening revealed the presence of carbohydrates, alkaloids, flavonoids, sterols, tannins, and triterpenes. Compatibility studies via FTIR demonstrated no significant interaction between eugenol and polymers used. Ten formulations (F1–F10) were developed and evaluated for physical characteristics, pH, spreadability, viscosity, extrudability, and drug content. Among these, formulation F8 exhibited optimal physicochemical properties with a pH of 4.4, good spreadability (5.5 g·cm/sec), high drug content (105.50%), and suitable viscosity (6400 cp). In-vitro release studies showed sustained drug release from F8 (71.56% at 360 minutes), while pure eugenol released 95.47% at 240 minutes. Antimicrobial efficacy of the optimized gel demonstrated a larger zone of inhibition (10 mm) against Candida albicans compared to the marketed clotrimazole gel (8 mm), indicating superior antifungal activity. Accelerated stability studies confirmed the formulation's stability over 30 days with negligible changes in key parameters. In conclusion, the optimized eugenol-based mucoadhesive gel (F8) offers promising potential as an effective and stable formulation for the management of vaginal fungal infections.
Keywords: Mucoadhesive Gel, Eugenol, Carbopol 934, Vaginal fungal infection, Antimicrobial activity, Physicochemical Properties.

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INTRODUCTION

Vaginal drug delivery presents several distinct advantages over traditional routes like oral or parenteral administration [1]. It bypasses hepatic first-pass metabolism and provides high permeability for various drugs, making it suitable for both local and systemic treatments. The vaginal mucosa, with its extensive surface area and rich blood supply, enhances drug absorption and therapeutic efficacy [2]. Recent studies have increasingly explored vaginal drug delivery systems as effective alternatives, particularly for minimizing side effects and enabling the non-invasive administration of sensitive molecules such as proteins and peptides [3].

Eugenol, a natural compound recognized for its antimicrobial, anti-inflammatory, and analgesic properties, holds promise for managing vaginal infections [4,5]. Formulating eugenol into a mucoadhesive gel may enhance its effectiveness and improve patient adherence [6]. The present study aimed to formulate and evaluate a mucoadhesive vaginal gel containing eugenol as the principal active agent for the treatment of vaginal fungal infections.

MATERIAL AND METHODS

Materials

Materials such as Carbopol 934, Sodium CMC, Tween 80, Methyl Paraben, and cellophane membrane were procured from SND College of Pharmacy, Babhulgaon, Yeola. The active pharmaceutical ingredient (API), Eugenol, was purchased from Oswal Chemicals, Chinchwad, Pune. All materials were of analytical grade and used as received without further purification.

Methods

Preformulation Study

Organoleptic Properties

The drug sample was observed for color, odor, and Appearance [6].

Solubility Analysis

Adding tiny amounts of solute to a predetermined volume of solvents, such as phosphate buffer, ethanol, chloroform, and acetone, and then monitoring for undissolved particles is known as solubility analysis [7].

pH determination

Eugenol is a slightly viscous liquid; it is typically diluted in distilled water to prepare a 1% w/v solution. Measure 1 mL of Eugenol and transfer it into a 100 mL beaker. Add 99 mL of distilled water to the beaker to make a 1% solution. Stir the mixture thoroughly using a glass rod. Rinse the pH meter's electrode with distilled water and gently blot dry with tissue paper. Immerse the electrode in the prepared Eugenol solution. Allow the pH meter to stabilize and note the reading [8].

Boiling Point of Eugenol

The capillary tube method was used to measure the boiling point of eugenol while maintaining a stirrer to ensure even heat transmission. Three separate readings were taken, and the mean value and the reported value were compared [7, 8].

Preliminary Phytochemical analysis

These extracts were tested in order to find out the presence of active compounds by use of following standard methods. The phytochemical screening of the plant extract revealed the presence of various secondary metabolites. Carbohydrates were confirmed by Molisch's (purple ring), Benedict's (orange precipitate), and Fehling's tests (reddish-brown precipitate). Proteins tested positive in Xanthoprotic (white precipitate) and Biuret tests (violet-pink color). Lipids were identified through solubility, glycerol, and Sudan III tests, with clear dissolution and red coloration. Alkaloids were detected using Mayer's, Wagner's, and Dragendorff's tests, all showing characteristic precipitates. Tannins were present as indicated by gelatin (curdy white), lead acetate (white precipitate), and ferric chloride tests (blue-green color). Saponins were identified by stable foam formation in the foam test. Flavonoids showed yellow coloration upon treatment with ammonia and sulphuric acid. Resins produced a violet color in the acetic acid-sulphuric acid test. Sterols were detected using the Salkowski test, indicated by a red chloroform layer. Cardiac glycosides (Keller Killiani test) showed a brown ring at the interface. Triterpenes were indicated by red coloration after heating with chloroform and sulphuric acid. Anthraquinones were confirmed by a pink ammoniacal layer after extraction with benzene and ammonia [7-10].

UV-Visible Spectrophotometry Analysis

Determination of λ Max of Eugenol

Calibration Curve of Eugenol

In a 100 ml volumetric flask, 10.0 mg of eugenol was dissolved in Tween 80. A 100 µg/ml stock solution was obtained by sonicating the solution and adding PBS pH 4.5 to bring it up to volume. To 10 µg/ml, this was further diluted. The λmax was calculated by scanning the solution between 200 and 400 nm. The concentrations of 5, 10, 15, 20, 25, and 30µg/ml were then achieved by diluting the stock solution. These solutions' UV absorbance at 279.2 nm was measured, and the absorbance value was used to plot a calibration curve [11-14].

Determination of Infrared Absorption Spectrum of Eugenol

The FTIR absorption spectrum of eugenol was recorded using the potassium bromide dispersion technique in an FTIR spectrophotometer [15, 16].

Fourier Transform Infrared Spectroscopy study

Drug-excipient interactions determine how much drug is released from formulations. Drug-excipient interactions are investigated using Fourier transform infrared spectroscopy. To determine the compatibility of eugenol and its combinations with polymers, FTIR spectroscopy was used. [17-20].

Formulation of Eugenol Gel

Water was treated with methylparaben as a preservative (Solution 1). Carbopol 934 and sodium CMC were dispersed in water and left to soak for 24 hours (Solution 2). Solution 1 was then mixed with Solution 2 to create Solution 3. Eugenol was added to Solution 3, and Triethanolamine was used to adjust the pH until a

viscous, homogeneous gel was obtained. The formulation code for the gel preparation is shown in the Table 1 [21-23].

Table 1: Composition of Gel Formulation

Formulation Code	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Drug (%)	1	1	1	1	1	1	1	1	1	1
Sod. CMC (%)	1.5	2.0	2.5	3.0	-	-	-	2.0	2.0	2.0
Carbopol 934 (%)	-	-	-	-	0.5	1.0	1.5	0.5	1.0	1.5
M.P (%)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
TRI	-	-	-	-	qs	qs	qs	qs	qs	qs
Tween 80 (%)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
D/W upto	100	100	100	100	100	100	100	100	100	100

Evaluation of Eugenol Gel

Evaluation of Eugenol gel formulations was evaluated for the following parameters.

pH

The pH of the gel formulations was measured using a calibrated pH meter [24].

Appearance and Clarity

The formulations were examined for suspended particle matter, color, and odor. The solutions were classified as turbid (-), slightly turbid (+), or clear and transparent (++) based on their clarity when compared to dark and white backgrounds [25].

Viscosity

A programmable viscometer (Brookfield, RVDV pro-II, USA) was used to measure the viscosity of premade gels at 37 °C. A Brookfield viscometer with a T-bar spindle was used to measure the gel's viscosity after the formulations had been equilibrated for 24 hours at 37 °C. Every ten seconds, the angular velocity was changed from 0.5 to 100 rpm, and the viscosity values at each rpm were recorded. For the same gel sample, I conducted the experiment three times and recorded the average reading [26].

Spreadability

On a glass plate, 0.5 g of each gel formulation was arranged in a circle with a diameter of 1 cm. A 500 g weight was applied for five minutes while another glass plate was on top. For comparison, the spread circle sizes were measured in centimeters [27].

Extrudability

Standard collapsible aluminum tubes were filled with the gel composition, sealed, and weighed. Next, the tubes were clamped between glass slides and a 500g weight was placed on top of them. To determine the percentage, the extruded gel was gathered and weighed once the cap was removed [28].

Mucoadhesive Strength

A modified balance assembly was used in a study to thoroughly assess the gel adhesion strength on goat vaginal mucosa. After being cleansed, the mucosa was preserved in a buffer solution. The force needed to separate the gel sample from the mucosa was measured using a lever equipped with vials and a pan [29].

Drug Content

After dissolving 1 g of eugenol gel in 100 ml of pH 4.5 phosphate buffer, the mixture was ultrasonically sonicated, agitated for an hour, and filtered. A UV spectrophotometer (Shimadzu, UV-1700, Japan) was used to measure the absorbance of the filtrate at 279.2 nm, using phosphate buffer at pH 4.5 as the blank. A calibration curve of eugenol in phosphate buffer at pH 4.5 was used to determine the drug content % [30].

Fourier Transform Infrared Spectroscopy (FTIR)

The drug and polymer samples made as KBr pellets were scanned throughout the 4000-400 cm-1 range using a Fourier Transformed Infrared Spectrophotometer (Shimadzu-8101A, Japan). All of the peaks in the FT-IR spectra of eugenol stayed the same when combined with the polymers Carbopol 934 and SOD CMC, according to the FTIR drug excipient compatibility research. This suggests that there is no interaction between the drug and the polymer, hence validating their compatibility [31-34].

Determination of *In-Vitro* Release of Drug from Formulated Gel

A Franz diffusion cell was used in a laboratory investigation to measure the release of eugenol. The donor and receptor compartments were separated by a cellophane membrane that had been soaked in PB pH 4.5. After applying 1g of gel to the membrane, 20 ml of PB pH 4.5 was added to the receptor compartment. At 37ºC, the receptor media was agitated. Using a UV spectrophotometer, samples were collected over the course of five hours and examined for eugenol at 279.2 nm. The calibration curve was used to compute cumulative percentages [35].

Antifungal Efficacy Studies

Using the Agar Diffusion Test, we evaluated the antifungal efficacy of optimized vaginal gel F8 against Candida albicans and contrasted it with Clotrimazole gel. Using the Hiantibiotic Zone Scale, we determined the zone of inhibition surrounding each cup and contrasted it with the reference [36].

Accelerated Stability Studies of Vaginal Gel

The gel formulation was prepared according to formula F8, filled in an aluminum collapsible tube, and stored at 40ºC, 2-8ºC, and room temperature. Samples were taken at 7, 15, and 30-day intervals for evaluation [37-39].

RESULTS AND DISCUSSION

Preformulation Study

Organoleptic Property

Table 2. displayed information about organoleptic properties and Table 3 showed boiling point of Eugenol.

Table 2: Organoleptic Properties

Test	Observation
Appearance	Pale yellow liquid
Odour	Aromatic odour
Color	Pale yellow
Taste	Pungent, Spicy

Boiling Point Determination

Table 3: Boiling Point of Eugenol

Sample	Reported	Observed
Eugenol	254ºC	252-254ºC

Preliminary Phytochemical analysis

Table 4(A&B) explained and showed result of the phytochemical analysis of eugenol

Table 4 (A): Result of the phytochemical analysis of Eugenol

Sr. No.		Phytochemicals Extracts				
			Aqueous	Methanol	Ethyl acetate	Petroleum ether
Primary metabolites						
1		**Carbohydrates**				
	a.	Molisch's test	-	+	-	-
	b.	Benedict's test	-	+	-	-
	c.	Fehling's test	+	-	-	-
2		**Protein**				
	a.	Xanthoprotic test	-	-	-	-
	b.	Biuret test	-	-	-	-

Table 4 (B): Result of the phytochemical analysis of Eugenol

Sr. No.		Phytochemicals Extracts	Aqueous	Methanol	Ethyl acetate	Petroleum ether
		Lipid				
	a.	Solubility test	-	-	-	+
	b.	Glycerol test	-	-	-	-
	c.	Sudan III test	-	-	-	-
Secondary metabolites						
1		**Alkaloids**				
	a.	Mayer's test	-	+	+	-
	b.	Dragendroff's test	-	+	-	-
	c.	Wagner's test	+	+	+	-
2		**Saponins**				
	a.	Foam test	-	-	-	-
3		**Flavonoids test**	-	+	+	-

4.		**Resins test**	-	-	-	-
5		**Tannins test**				
	a.	Gelatin test	-	+	+	-
	b.	Lead Acetate test	-	+	+	-
	c.	Ferric chloride test	+	+	+	-
6.		**Sterols**				
	a.	Salkowski test	+	+	+	-
7		**Cardiac Glucosides**				
	a.	Keller – Killiani test	-	-	-	-
8		**Triterpenes test**	-	+	+	-
9		**Anthraquinones test**	-	-	-	-

Note: **(+)** represents the presence of the constituents **(-)** represents the absence of the constituents

Solubility Analysis

Table 5 illustrated information about solubility analysis study.

Table 5: Solubility Analysis

Solvents	**Solubility Behaviors**
Ethanol	Miscible
Phosphate buffer	Soluble
Water	Slightly soluble (0.2 g/100ml)
Chloroform	Miscible
Acetone	Miscible
Ether	Miscible

pH of Solution

The pH value of Eugenol was recorded as 5.8.

UV Spectroscopic study for Eugenol (Determination of λ max)

The maximum absorption wavelength (λ max) of Eugenol in PBS pH 4.5 was found to be 279.2 nm as shown in Table 6 and Figure 1.

Table 6: Wavelength of maximum absorption (λ max)

Solvent	**λ max (nm)**
PBS pH 4.5	279.2

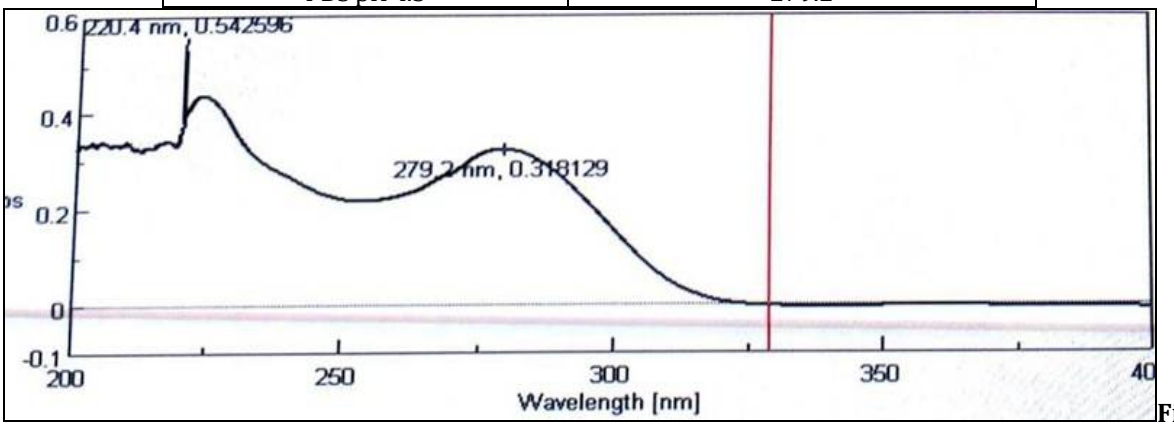

Figure 1: Wavelength of maximum absorption (λ max)

Preparation of Standard Curve of Eugenol

PBS was used to create eugenol solutions with pH 4.5 at concentrations ranging from 5 to 25. Each solution's absorbance was determined using a Shimadzu UV-Visible double-beam spectrophotometer with PBS at 279.2 nm. To find the regression equation and correlation coefficient, a typical absorbance versus concentration plot was made as shown in Table 7 and Figure 2.

Table 7: Calibration Curve in Phosphate Buffer Solution (pH 4.5)

Concentration(µg/ml)	Absorbance at 279.2 nm
0	0.00
5	0.0523
10	0.1397
15	0.241
20	0.3258
25	0.4323

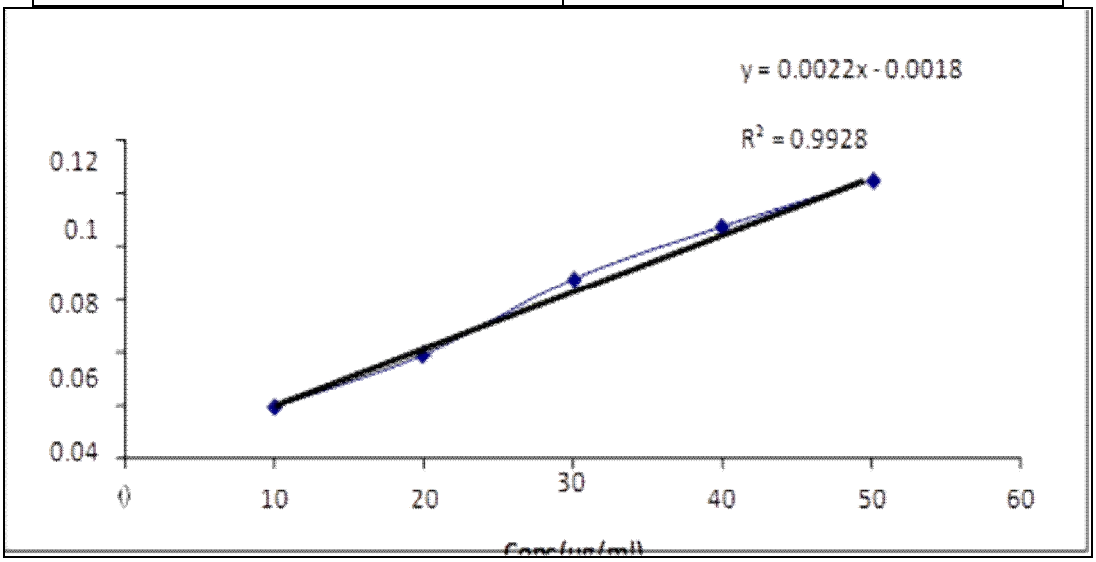

Figure 2: Calibration Curve in Phosphate Buffer Solution (pH 4.5)

Compatibility studies

FTIR study

FTIR studies were performed to evaluate potential interactions between the drug and polymers. The spectra of Eugenol and its mixtures with polymers are shown in Figures 3-6 and Table 8. The FTIR spectra of Eugenol displayed peaks that remained mostly unchanged, with only slight shifts, indicating that there were no significant interactions with the polymers and the drug maintained its stability. The key functional groups observed in the spectra include -OH, -C-H (alkyl), -C=C- (aromatic), -OCH3, and -C=C- as detailed in Table 8.

Table 8: IR spectrum Interpretation (Wave number and respective functional group)

Wave number (cm-1)	Functional group
3517	-OH
2983	-C-H (Alkyl)
1608	-C=C- (Aromatic)
1440	-OCH3
910	-C=C-

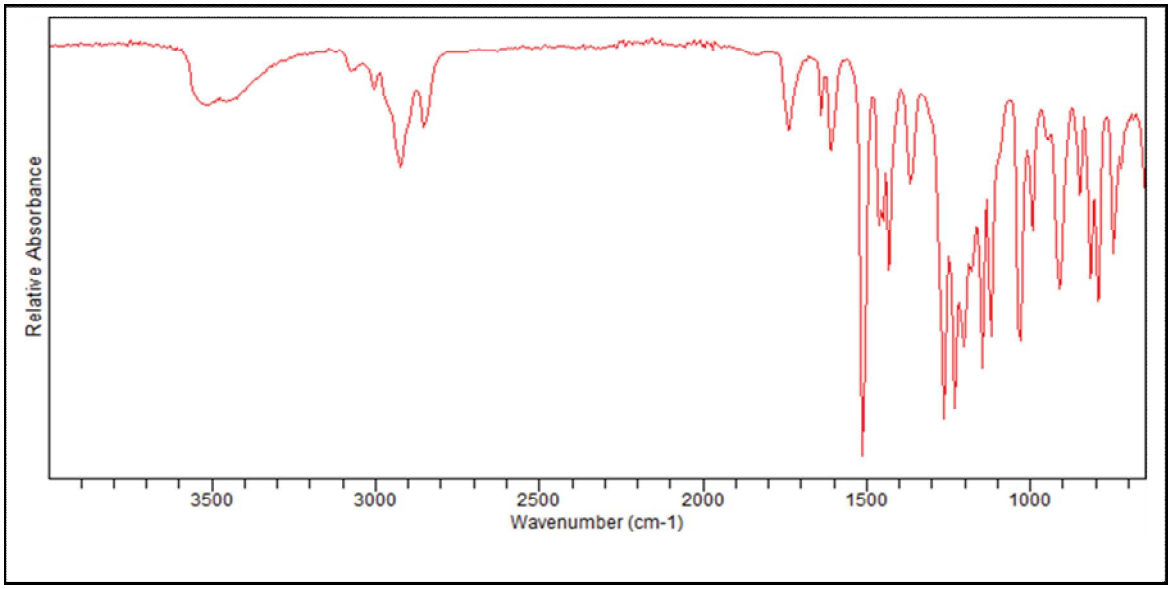

Figure 3: FTIR of Eugenol

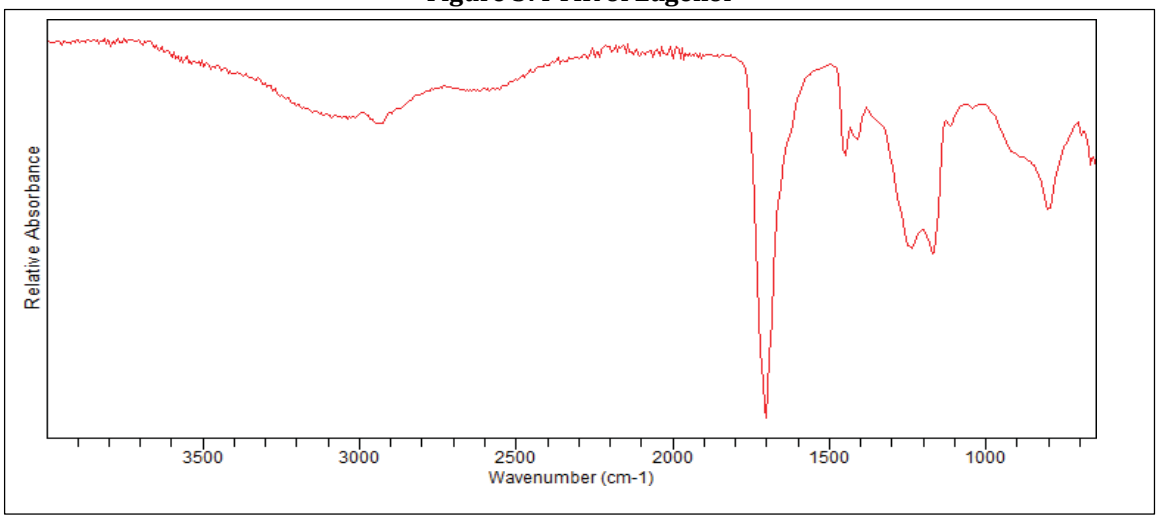

Figure 4: FTIR of Carbopol 934

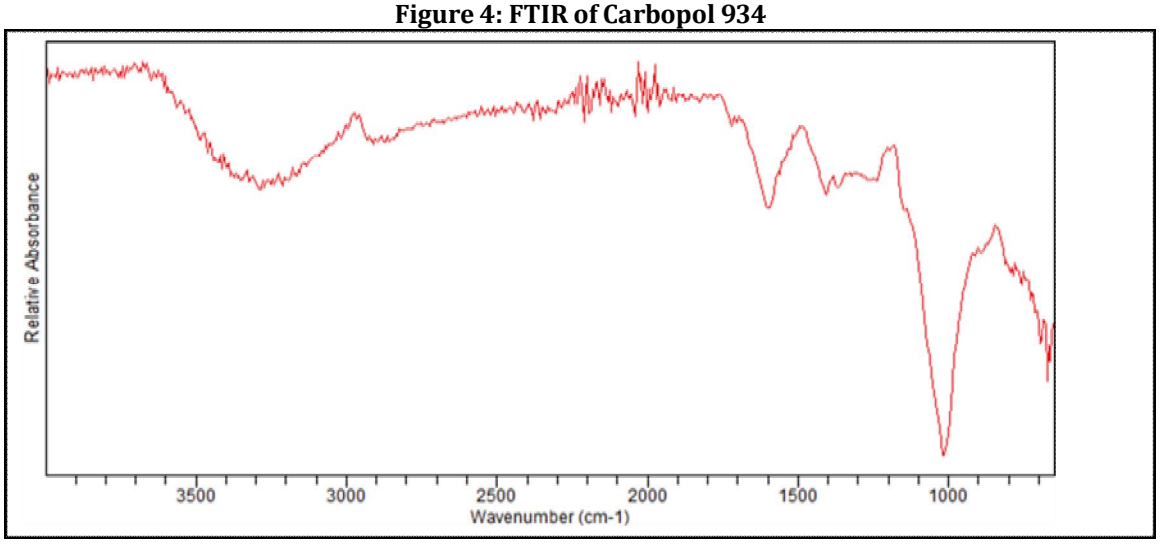

Figure 5: FTIR of Sodium CMC

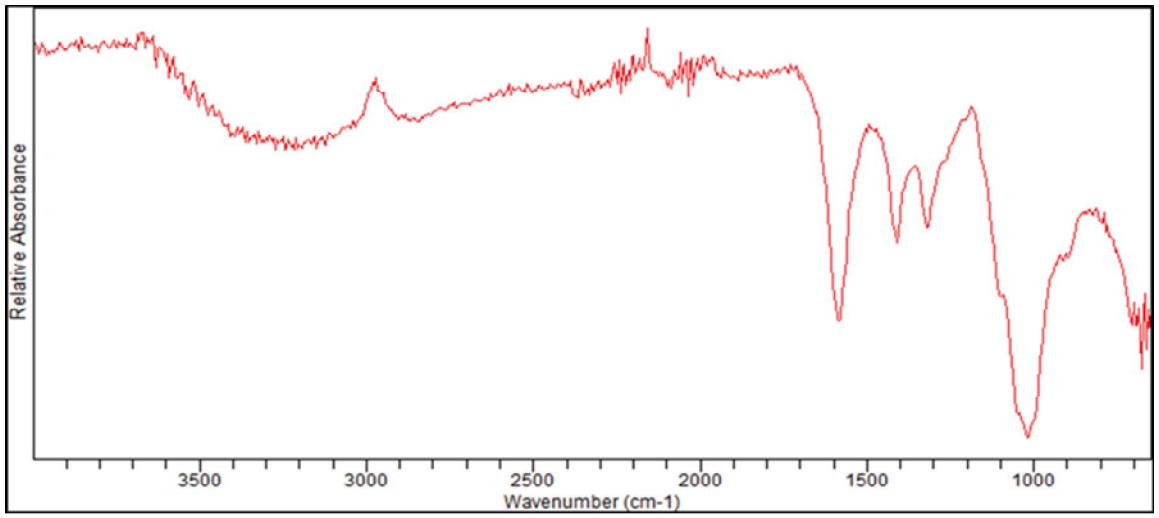

Figure 6: FTIR of Xanthan gum

Characterization of Gel Formulation

Table 9 presents the physical properties of the gel formulations.

Table 9: Indicates Physical Characteristics of Gel Formulations

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Appearance	++	++	++	++	++	+	+	++	+	+
pH	7.2	7.04	7.0	6.9	4.6	4.7	4.7	4.4	4.6	4.7
Spreadability	7.0	6.4	5.7	5.2	5.8	3.6	3.3	5.5	3.5	3.1
Net Content (%)	96.28	96.20	102.70	98.30	105.40	104.90	96.97	105.50	103.90	102.94
Extrudability (%)	89.53	86.8	75.58	69.50	76.92	45.97	43.75	80.00	45.70	43.90

Appearance, Clarity, and pH

Every gel formulation was transparent and clear. The pH values of the compositions varied from 4.4 to 7.0. It was appropriate for vaginal use since the vaginal cavity is acidic.

Net content

96 to 105%

Spreadability

The spreading qualities of the formulation determine the effectiveness of local or topical therapy. Table 9 shows that the spreadibility of the gel formulation at 37°C dramatically reduces as the concentration of mucoadhesive agent increases because of the increased viscosity

Viscosity

The viscosity of mucoadhesive vaginal gels is crucial for their retention and spreading in the vagina. Higher viscosities help minimize seepage, which can cause discomfort to the patient. Increasing the polymer concentration led to a corresponding increase in viscosity for Na CMC and Carbopol 934 vaginal gels as shown in Table 10.

Table 10: Viscosity of the Gel Formulations

Formulation Code	Viscosity of gels formulations at 100 RPM at 31°C (CP)
F1	550
F2	806
F3	1200
F4	1700
F5	6800
F6	7560
F7	9800
F8	6400
F9	7140
F10	9300

***In-vitro* drug release**

The in-vitro drug release profile shows that pure Eugenol (API) exhibited a faster release, reaching 95.47% at 240 min, while formulation F8 showed a sustained release pattern, reaching 71.56% at 360 min as shown in Table 11.

Table 11: Data for *In-vitro* Drug release study

Formulation	Time(min)							
	15	30	60	120	180	240	300	360
Eugenol (API)	8.44	19.83	36.5	56.75	75.53	95.47	88.07	77.88
F8	12.04	17.44	25.72	34.34	45.07	56.47	62.2	71.56

Mucoadhesive strength

The property determines how long the formulations stay in the vaginal cavity. It is mainly affected by the self-cleansing action & involves electrostatic interactions or hydrogen bonds with the carbohydrate branches of the mucin.

Antimicrobial Efficacy Studies:

The antifungal activity of the optimized batch was compared with the marketed Clotrimazole gel using the Cup and Plate technique. The optimized batch showed a greater zone of inhibition compared to the marketed formulation as shown in Table 12 and 13 along with Figures 7 and 8.

Antifungal Activity Drug Concentration

Table 12: Antifungal activity of eugenol

Quantity of sample (µl)	Zone of Inhibition of Candida albicans (Mm)
20	7
40	9
60	6
80	9
20(S)	10

Figure 7: Zone of inhibition for drug concentration against candida albicans

Table 13: Antifungal activity of optimized vaginal gel with marketed clotrimazole gel

Parameter	Zone of inhibition of candida albican(Mm)
Marketed preparation	8
Optimized gel formulation	10
Standard concentration on well	12

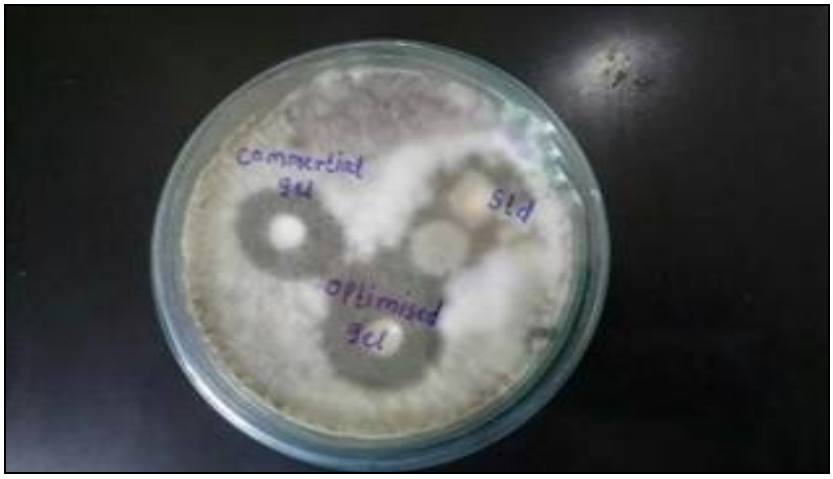

Figure 8: Comparison of antibacterial activity of Optimized vaginal gel with marketed Clotrimazole gel formulation

Accelerated Stability Studies

The formulation remained stable over 30 days with no significant changes in appearance, color, or odor, while slight variations were observed in pH, viscosity, spreadability, and assay under different storage conditions as shown in Table 14.

Table 14: Stability Studies

Sr. N o	Parameter	Initial	Duration 7 days		15days		30days	
1	Temp(ºC)	R. T.	8ºC	40ºC	8ºC	40ºC	8ºC	40ºC
2	Appearance	Semisolid						
3	Color	Clearly						
4	Odor	Characteristics						
5	pH	4.4	4.4	4.4	4.5	4.5	4.5	4.5
6	Viscosity	22200	22100	22050	21900	22840	21860	21780
7	Spreadability	5.5	5.5	5.5	5.6	5.6	5.7	5.7
8	Assay	105.50	104.40	104.30	103.80	103.40	103.10	102.90

CONCLUSION

The present study successfully developed and evaluated a mucoadhesive vaginal gel containing eugenol, which demonstrated significant potential in the treatment of vaginal fungal infections. The preformulation and phytochemical analyses confirmed the identity, purity, and presence of key bioactive constituents in eugenol. Among the various formulations, F8 was found to be the most effective, exhibiting optimal physicochemical properties such as appropriate pH (4.4), good spreadability, high drug content, and suitable viscosity for vaginal application. FTIR studies indicated no major drug-polymer interactions, ensuring the stability of the formulation. In-vitro drug release studies revealed that F8 provided a sustained release profile, enhancing its therapeutic potential. The antimicrobial study showed that the optimized gel exhibited a greater zone of inhibition against *Candida albicans* than the marketed clotrimazole gel, confirming its superior antifungal efficacy. Stability studies further validated the formulation's physical and chemical stability over 30 days under various storage conditions. Overall, the eugenol-based mucoadhesive gel formulation F8 proved to be a promising, safe, and effective alternative for the treatment of vaginal fungal infections, warranting further clinical investigation.

REFERENCES

1. Fusan A, Carturk H. (2009) Mucoadhesive Vaginal Drug Delivery Systems. Journal of Recent Patents on Drug Delivery & Formulation.3:193-205.
2. Dobaria N, Mashru R, Vadia N. (2007) Vaginal Drug Delivery Systems: A Review of Current Status. East and Central African Journal of Pharmaceutical Sciences.10:313-318.
3. Boddupalli, B. M., Mohammed, N. K., Nath, R. A., & Banji, D. (2010). Mucoadhesive drug delivery system: An overview. Journal of Advanced Pharmaceutical Technology & Research, 1(4), 381-387.
4. Khan, A. B., Mahamana, R., & Pal, E. (2014). Review on mucoadhesive drug delivery system: Novel approaches in modern era. Journal of Pharmaceutical Science, 4, 128-141.
5. Dyah N, Murika A, Bramanti I, Wardani PK. (2024). Effectiveness of Eugenol Treatment on IL-6 Expression in

Pulpitis. J Med Chem Sci.; 7:1216–24.
6. Pramod, K., Ansari, S. H., & Ali, J. (2010). "Eugenol: A Natural Compound with Versatile Pharmacological Actions." Natural Product Communications, 5(12), 1999-2006.
7. Prabhat Desai, Gauri M. Mhaskar. (2019) Formulation and Evaluation of *Zingiber officinale* Emulgel. Research J. Pharm. and Tech.12(3): 1294-130.
8. Rizwana, H., & Dhanaraju, M. D. (2018). "Standardization and Determination of Boiling Point of Eugenol from *Syzygium aromaticum*." Journal of Pharmacognosy and Phytochemistry, 7(1), 135-140.
9. Zahoor Ahmad Lone,Navin Kumar Jain.(2022). Phytochemical analysis of clove (*Syzygium aromaticum*) Dried Flower Buds Extract and its therapeutic importance 12[4-S]:87-92
10. Chinala KM, Chaithanya A, Sawrov M, Sahithi A, Achyuth C. (2025). Phytochemical and Antimicrobial Evaluation of *Laurus nobilis* Leaves Against Acne and Dandruff-Causing Microorganisms. J Pharm Sci Comput Chem.;1(1):50–7.
11. Grubbs, W. T. (2010). "The Capillary Method for Boiling Point Determination of Organic Liquids." Journal of Chemical Education, 87(3), 307-309.
12. Hossain, M.A., & Al-Toubi, W (2011). Spectroscopic investigation of eugenol: Effect of solvents and medium. Journal of Pharmaceutical Sciences, 100(4), 1307–1315.
13. Godse SD, Sawant SD. (2024). Development and Validation of Stability-Indicating HPTLC Method for Mirabegron with Identification, and Characterization of Degradant by ESI-MS. Asian J Green Chem.;8(4):397–410.
14. Patel J, Tandel J, Chhalotiya U, Patel K. (2021). Stability indicating planar chromatographic method for estimation of minoxidil and finasteride combination used in the treatment of hair loss. J Med Chem Sci.;4(1):17–28.
15. Mishra, A., & Sharma, R. (2014). UV-visible spectrophotometric analysis of eugenol in pharmaceutical formulations. International Journal of Pharmaceutical Research, 6(2), 150-15
16. El-Shahat, M.F., Hassan, H.H., & El-Sayed, M.M. (2012). FTIR Spectroscopic Analysis of Eugenol and Its Derivatives. Journal of Applied Spectroscopy, 79(5), 773-780.
17. Lopes, G.K., Schulman, H.M., & Hermes-Lima, M. (2002). Spectroscopic analysis of eugenol and its structural analogs using FTIR. Journal of Molecular Structure, 606(1-3), 119-126.
18. Prakash, K., & Goli, D. (2014). Drug-Excipient Interactions: Case Studies and FTIR Spectroscopic Analysis. International Journal of Pharmaceutical Sciences and Research, 5(2), 54-62.
19. Ali, H.H., & Abbas, Z.S. (2018). Compatibility and Interaction Studies of Eugenol and Excipients by FTIR Spectroscopy and Thermal Analysis. Journal of Drug Delivery Science and Technology, 44, 238-245.
20. Alhussein ABA, Gaaz TS, Jaaz AH, Alsultany FH, Kadhum AAH, Al-Amiery AA, et al. (2025). Preparation of Nanoparticles Loaded by Dimethyl Fumarate and Their Physical and Chemical Properties Study. Adv J Chem Sect A.;8(1):194–208.
21. Rachmawati, H., Alisjahbana, I.A., & Yoshioka, S. (2013). Compatibility Studies of Eugenol and Its Polymer Excipients by FTIR Spectroscopy. Journal of Pharmaceutical and Biomedical Analysis, 81, 92-98.
22. Sharma, A., & Shrestha, S. (2018). Formulation and Evaluation of Topical Eugenol Gel for Its Antibacterial Activity. Journal of Drug Delivery Science and Technology, 45, 270-276.
23. Saini, R., Sharma, S., & Singh, G. (2017). Formulation, Development and Evaluation of Eugenol Gel for Antimicrobial Activity. International Journal of Pharmaceutical Sciences and Research, 9(3), 912.
24. Silva, J.A., Souza, M.A., & Rocha, M.A. (2016). Rheological Behavior of Carbopol-Based Gels: Influence of Polymer Concentration and Neutralizing Agent. Journal of Applied Polymer Science, 133(13),
25. Fadhl, B. M., & Al-Farabi, A. S. (2021). Evaluation of pH, viscosity and thermal stability of a gel formulation. Journal of Pharmaceutical Sciences, 110(5);1951-1960.
26. Adhikari, R., & Sadhu, S. K. (2019). Formulation and characterization of gel containing herbal extracts. Journal of Drug Delivery and Therapeutics, 9(1), 360-365. https://doi.org/10.22270/jddt.v9i1.2397Desai K, et al. Evaluation of Extrudability of Eugenol Gel Formulations. Journal of Drug Delivery Science and Technology. 2021; 61:102197. doi: 10.1016/j.jddst.2021.102197
27. Choudhary, M. I., & Raza, M. (2017). Viscosity measurements of liquid formulations: A comprehensive overview. Pharmaceutical Sciences, 23(3), 209-214.
28. Singh, A., & Raghavendra, S. (2015). Formulation and evaluation of herbal gel. International Journal of Pharmaceutical Sciences and Research, 6(5), 2024-2030.
29. Desai K, et al. (2020) In Vitro Drug Release and Spreadability of Eugenol-Containing Gels. Asian Journal of Pharmaceutical Sciences.15(5):606-616.
30. Al-Shahrani, S. A., & Khan, M. I. (2020). Development and characterization of hydrogels for drug delivery systems.Journal of Drug Delivery Science and Technology, 55.
31. Jain, S. K., & Tiwari, A. (2017). Development and evaluation of mucoadhesive gel for vaginal delivery of antifungal agents. Journal of Drug Delivery Science and Technology, 41, 241-247.
32. Sruthi V, Shankar Rao GB. (2024). Extraction, Phytochemical Screening, and Isolation of Active Fraction of Turnera ulmifolia Linn. Asian J Green Chem.;8(4):411–26.
33. Ashindortiang OI, Anyama CA, Ayi AA. (2022). Phytosynthesis, Characterization and Antimicrobial Studies of Silver Nanoparticles Using Aqueous Extracts of *Olax Subscorpioidea*. Adv J Chem Sect A.;5(3):215–25.
34. Salman SS. (2024). Green Synthesis, Analysis, and Characterization of Nano-silver- Based Conyza Canadensis (SYN: Erigeron Canadensis) Extract. Chem Methodol.;8:856–73.
35. Nascimento, J. R. O., & Freitas, M. C. (2020). Development and characterization of Eugenol loaded gels. Brazilian

Journal of Pharmaceutical Sciences, 56(1), e17120.
36. Nayak, A. K., & Ghosh, A. (2015). Compatibility study of eugenol and various excipients by FT-IR spectroscopy. International Journal of Research in Pharmaceutical Sciences, 6(3), 224-227.
37. Chourasia, M. K., & Jain, S. K. (2016). In vitro release study of various mucoadhesive drug delivery systems. Journal of Drug Delivery Science and Technology, 31, 1-8.
38. Arora, D. S., & Kaur, J. (2016). Antifungal activity of clotrimazole and eugenol against clinical isolates of Candida species. Journal of Mycology, 2016, 1-8.
39. Dhiman, A., & Jangra, A. (2019). Formulation and stability studies of topical gel formulations: A review. International Journal of Pharmaceutical Sciences and Research, 10(2), 573-584.