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ORIGINAL ARTICLE

Design and Characterization of *Allium sativum* Extract-Loaded Organogel for Antifungal and Antibacterial Activity

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ABSTRACT

The present study focuses on the development and evaluation of a topical organogel formulation containing Allium sativum (garlic) extract for antibacterial and antifungal applications. Allium sativum, known for its broad-spectrum antimicrobial properties, was extracted using a hydroethanolic solvent system and characterized using UV-Vis spectroscopy, FTIR, and HPLC, confirming the presence of allicin and other bioactive organosulfur compounds. The extract was incorporated into an organogel base formulated using lecithin, oleic acid, and other excipients. A Box-Behnken design was employed to optimize the formulation parameters, targeting viscosity and spreadability as critical quality attributes. The optimized organogel batch exhibited desirable physicochemical properties including suitable pH (6.25), high drug content (91.28%), good spreadability (24.3 g.cm/sec), and stable viscosity (3200 cP). In vitro antimicrobial studies demonstrated significant inhibition zones against Staphylococcus aureus and Candida albicans. Drug release kinetics followed the Korsmeyer-Peppas model, suggesting diffusion-controlled release. The formulation remained stable over one month without significant changes in key parameters. These findings suggest that Allium sativum organogel holds promise as an effective and natural topical treatment for bacterial and fungal infections.

Keyword: *Allium sativum*, Allicin, Topical drug delivery, Phytochemical screening, Box-Behnken design, Herbal formulation, Drug release kinetics

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INTRODUCTION

A targeted medication delivery method that can be applied topically through the skin, vagina, rectal, or ocular channels is known as topical drug administration. The skin serves as the primary channel for topical medication delivery and is one of the most accessible organs on the human body for topical administration. Applying topical treatments to the skin might have systemic, local, or superficial effects. Because of its emollient, calming, or protective qualities, the base may occasionally be used on its own.[1] The use of chemical enhancers, biopolymers (such as sodium hyaluronate), liposomes, particulate carriers (microspheres and lipid nanoparticles), topical sprays and foams, occlusion (through dressings and patches), topical peels, temperature (heat), iontophoresis, and ultrasound are some of the current

and emerging methods for optimizing the topical delivery of dermatological agents (small and large molecules).[1]

The skin and its descendant components make up the integumentary system. The epidermis, dermis, and subcutaneous tissue are the three layers that make up the skin. The outermost layer, the epidermis, is made up of a particular kind of cells called keratinocytes, which are responsible for producing the protective protein keratin, which is long and thread-like. Collagen is essentially the fibrillary structural protein that makes up the middle layer, or dermis. The dermis is located on the panniculus, or subcutaneous tissue, which has tiny lobes of lipocytes, which are fat cells. Depending on the body's anatomy and geographic location, these layers' thicknesses vary significantly. For instance, the epidermis layer on the eyelid is the thinnest, measuring less than 0.1 mm, while the epidermal layer on the palms and soles of the feet is the thickest, measuring about 1.5 mm. The back has the thickest dermis, which is 30–40 times thicker than the epidermis on top.[2]

Organogel is a thermoreversible (thermoplastic) solid that is non-crystalline and non-glassy. It is a semi-solid preparation with an external apolar phase that is immobilized. Because of the physical interactions between the self-assembled structures of compounds identified as gelators, the apolar phase becomes immobilized within the spaces of the three-dimensional networked structure. Frequently, these systems rely on the molecules' ability to self-assemble. Sterol, sorbitan monostearate, lecithin, and derivatives of cholesterol anthraquinone are a few typical examples of gelators. The organogels' thermo-reversible characteristic has led to the possibility of using them as a drug delivery system. The spontaneous development of a fibrous structure, which allows the organogels to exist in a low energy state, has been credited with giving them their thermodynamic stability. The fact that the gel-to-sol transition occurs at room temperature suggests that the organogels require external energy to break their three-dimensional structure and change from the gelled state to the sol state.[3]

Mechanism of Organogelation

The presence of a polar solvent causes lecithin molecules to gel. In the presence of a nonpolar media, lecithin has a tendency to self-assemble into reverse spherical micelles at concentrations of approximately 0.01 mM.[4]

Fluid-filled fiber mechanism

First, a mixture of surfactants and co-surfactants was dissolved in an apolar solvent, resulting in the formation of reverse micelles. When water was added, tubular reveres micelles were created. When water is added to tubular reverse micelles, the elongated micelles become trapped and form a three-dimensional network that immobilizes the apolar solvent.[5]

Solid Fiber Mechanism

After heating the solid organogelator and apolar solvent, an apolar solution of the organogelator was formed. When the organogelator cools to ambient temperature, it precipitates out as fiber that physically interacts with one another to create three-dimensional network structures that immobilize apolar solvent.[6]

Hydration Method

The inorganic chemicals can be directly hydrated to create a dispersed phase of dispersion, which is how gel is made. To improve gel formation, other substances such as propylene glycol, propyl gallate, and hydroxyl propyl cellulose may be utilized in addition to water vehicles.[6]

Plant Profile

Garlic (*Allium sativum*)

The bulbous plant known as garlic can grow up to 1.2 meters in height. Garlic is easy to grow and does well in moderate climates. Two of the many types or subspecies of garlic are hard neck and soft neck. Allicin, sometimes referred to as diallyl thiosulfinate or allyl 2-propenethiosulfinate, is the primary bioactive component of raw garlic homogenate or garlic aqueous extract. When garlic is chopped or crushed, the enzyme alliinase, which is found in intact garlic, is activated, converting alliin into allicin. y-L-glutamyl-S-alkyl-L-cysteine, (E, Z)-4,5,9-trithiadodeca-l,6,11-triene 9-oxide (ajoene), allyl methyl thiosulfonate, and 1-propenyl allyl thiosulfonate are also present in garlic homogenate. The concentration of adenosine increases several times as the homogenate is incubated at room temperature for a few hours.[7]

Figure. 1 *Allium sativum* Plant [8]

Table. 1 Taxonomical Classification of *Allium sativum*[9]

Kingdom	Plantae
Subkingdom	Trachebionta
Division	Tracheophytes
Subdivision	Spermatophyta
Class	Liliopsida
Subclass	Asteridae
Order	Asparagales
Family	liliaceae
Genus	Allium

MATERIAL AND METHODS

Collection of plant material and chemicals

Allium sativum plant material was collected in sacks from Satana, Nashik District, Maharashtra, India. The plant material was authenticated by the Department of Botany, Loknete Vyankatrao Hiray Arts, Science and Commerce College, Panchavati, Nashik (M.S.), which is an affiliated body of Savitribai Phule Pune University. The authentication reference number is LVH/PG/BOT/807/22/11/2024, dated 22/11/2024. Soya lecithin, Oleic acid, Tween 80, Triethanolamine, Mentha oil, Sodium benzoate, Ethanol were purchased from Modern lab. Nashik.[10]

PREFORMULATION STUDY

Procedure for extraction of *Allium sativum* powder using Soxhlet apparatus

Powdered *Allium sativum* was put into a filter paper bag and put into a Soxhlet extractor. After adding a 20:80 v/v hydroethanolic solvent (Water: Ethanol), the round-bottom flask was gradually heated. After 72 hours of repeated cycles, the solvent vapors condensed and removed phytochemicals from the powder. To create a brown, semisolid crude extract, the extract was then concentrated using a rotary evaporator at lower pressure. It was then kept for later use at 4°C in a sterile, airtight container.[10]

Preliminary characterization of *Allium sativum* extract

Allium sativum extract was estimated for its organoleptic characteristics, solubility study, moisture content, ash content, FTIR, UV spectra and HPLC study by comparing the obtained result with standard.[11, 12, 13]

Identification of active constituents by phytochemical screening

Preliminary phytochemical screening was performed to identify the primary and secondary metabolites like flavonoids, alkaloids, saponins, carbohydrates, phenolic compounds, proteins etc., *Allium sativum* mainly contains the sulphur components so two sulphur tests were carried out i.e. sulphur test / lead acetate test and sodium nitroprusside test.[14, 15]

UV-Vis Spectroscopy

Allium sativum extract in phosphate buffer (pH 7.4) was measured for its UV spectrum between 200 and 400 nm. At 240 nm, the wavelength at which the maximum absorbance (λmax) was measured, the standard calibration curve was prepared. Calibration curve of *Allium sativum* Extract was constructed in Phosphate buffer pH 7.4.[16]

FTIR Spectroscopy

FTIR spectroscopy was used to identify functional groups in the ethanolic extract of *Allium sativum*. The extract was mixed with potassium bromide (KBr), compressed into a pellet, and analyzed using a Shimadzu FTIR 8400s spectrophotometer over the 4000–400 cm^{-1} range. The spectrum revealed characteristic absorption peaks indicating the presence of specific chemical bonds in the phytochemicals.[17]

Drug-Excipient Compatibility

To assess compatibility, the drug and excipients were mixed in a 1:1 ratio and sealed in amber glass vials. These were stored at 40 °C for 1 day, while control samples were kept at room temperature. After incubation, both sets were analyzed using FTIR spectroscopy to detect any possible interactions between the drug and excipients.[18]

HPLC (High Performance Liquid Chromatography)

High performance liquid chromatography used in separating the complex mixture of molecules encountered in chemical and biological system. The mobile phase composed of Methanol and water. The composition of mobile phase (70:30). The Flow rate is 0.8 ml/min. the wavelength is 254 nm. [19]

Table.2 HPLC Model

Model	**Shimadzu Prominence**
Company	Shimadzu analytical (India) Pvt. ltd
Pump	LC-20AD UFLC (40MPa)
Column	Cosmosil C18 (250MM,4.61ID, Particle size: 5micron)
Detector	SPD-20A
Software	LC Solution

MICROBIOLOGICAL STUDY

Minimum Inhibitory Concentration (MIC)

Antibacterial activity

MIC was determined using the disc diffusion method against *Staphylococcus aureus*. MHA plates were inoculated with the bacterial suspension, and sterile discs (6 mm) impregnated with various concentrations of *Allium sativum* extract (6 to 0.37 mg/ml) were placed on the agar. After 24 hours of incubation at 37 °C, the zones of inhibition were measured. The lowest concentration showing a clear inhibition zone was recorded as the MIC.[20]

Antifungal activity

MIC against *Candida albicans* was evaluated using the disc diffusion method on SDA plates. A standardized fungal suspension was spread on the agar, and sterile discs (6 mm) containing various concentrations of *Allium sativum* extract (12 to 0.37 mg/ml) were placed on the surface. Plates were incubated at 35 °C for 48 hours. Zones of inhibition were measured, and the lowest concentration showing a clear zone was recorded as the MIC.[20]

FORMULATION AND DEVELOPMENT OF ORGANOGEL

Experimental Design: Optimization Using Box-Behnken Design (BBD)

The application of a statistical experimental design to pharmaceutical formulation development has been demonstrated to be efficient and satisfactory in acquiring the necessary information to understand the relationship between independent and dependent variables in a formulation. The Box–Behnken design (BBD) is a divergent type of RSM (response surface methodology) design available for statistical optimization of formulations. This design offers a far more effective method than the conventional techniques of dosage form optimization because it involves many trial runs and less time. BBD strives to optimize the output of a system with the fewest runs. Three-factor, three-level Box–Behnken design is one of best-suited optimization techniques of response surface methodology.[21]

Batch optimization

In the current study, three formulation variables (factors) were selected based on preliminary screening studies and two critical quality attributes (responses) were evaluated.[22]

Table. 3 Parameters of Experimental Design

Factors	**Range**	
	Low Limit	**High Limit**
X1: POLYMER (% w/v)	2.5	10
X2: SURFACTANT (% v/v)	5	7.5
X3: pH ADJUSTER	0.4	0.6
Response	**Goal**	
Y1: Viscosity (cP)	In range	
Y2: Spreadability (g.cm/sec)	Maximum	

Table.4 Formulation Batches

Name	*Allium sativum* Extract (gm)	Soya Lecithin (gm)	Oleic acid (ml)	Tween 80 (ml)	Triethanolamine (gm)	Sodium benzoate (gm)	Mentha Oil	Water (ml)	Total quantity (gm)
F1	0.030	0.75	5	2.25	0.15	0.015	0.01	q.s.	20
F2	0.030	3	5	1.875	0.12	0.015	0.01	q.s.	20
F3	0.030	1.875	5	1.875	0.15	0.015	0.01	q.s.	20
F4	0.030	1.875	5	1.875	0.15	0.015	0.01	q.s.	20
F5	0.030	1.875	5	2.25	0.12	0.015	0.01	q.s.	20
F6	0.030	1.875	5	1.5	0.15	0.015	0.01	q.s.	20
F7	0.030	0.75	5	1.875	0.18	0.015	0.01	q.s.	20
F8	0.030	3	5	2.25	0.15	0.015	0.01	q.s.	20
F9	0.030	3	5	1.5	0.15	0.015	0.01	q.s.	20
F10	0.030	1.875	5	1.875	0.15	0.015	0.01	q.s.	20
F11	0.030	3	5	1.875	0.18	0.015	0.01	q.s.	20
F12	0.030	0.75	5	1.875	0.12	0.015	0.01	q.s.	20
F13	0.030	0.75	5	1.5	0.18	0.015	0.01	q.s.	20
F14	0.030	1.875	5	2.25	0.18	0.015	0.01	q.s.	20
F15	0.030	1.875	5	1.875	0.15	0.015	0.01	q.s.	20
F16	0.030	1.875	5	1.875	0.15	0.015	0.01	q.s.	20
F17	0.030	1.875	5	1.5	0.12	0.015	0.01	q.s.	20

Method of preparation of *Allium sativum* Extract Organogel

In gelation process takes place with addition of amount of water in the solution of polar solvent and surfactant like lecithin molecule. Before addition of water firstly prepare the surfactant dispersed in the organic medium then addition the small amount of water. The surfactant molecule assembles themselves in the form of micelles. Further the addition of water then makes short tubular or cylindrically micellar aggregates. Water molecules is bind stoichiometrically to the hydrophilic head of the molecules. One water molecule is linked with two surfactant molecules this forms a linear network with hydrogen molecule bonds between the phosphate group of lecithin molecule and polar molecule. In further addition of little amount of water in which the result is formation of worm like and flexible tubular micellar structure. The tubular micellar micro structure is formed interwine and overlap with other formed by 3-D gel fibres and fibrils network, which possesses viscoelasticity and thermo-reversibility properties.[23]

EVALUATION PARAMETER OF ORGANOGEL

Organoleptic characteristics

The prepared organogel formulation was inspected visually for its colour, homogeneity, consistency, grittiness, texture and phase separation.[24]

Determination of pH

The pH of the gel formulation has been analysed using a Digital pH meter and results were mentioned in table.[24]

Viscosity

The measurement of the viscosity of the prepared Organogel was done by using Brookfield viscometer (Brookfield DV-II+ Pro viscometer). The viscosity was measured using spindle no.T-bar D 94 at 12 rpm and at room temperature. A sufficient quantity of gel was filled in the wide-mouth container in such a way that it should sufficiently allowed to dip the spindle of the viscometer.[25]

Spreadability

One of the criteria for organogel to meet the ideal qualities is that it should possess good spreadability. It is a term expressed to denote the extent of area to which gel readily spreads on application to the skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading coefficient.[25]

$$Spreadability\ (S) = \frac{Weight(M)\ x\ Distance\ (L)}{Time\ (T)}$$

Drug content

The drug content was calculated using the linearity equation Y (absorbance) = 0.0195X + 0.022 for *Allium sativum* extract. The practical yield (PY) was compared with the theoretical yield (TY = 15 µg/mL), and the percentage drug content was determined using the formula[26]

$$\text{Drug Content } (\%) = \frac{(Practical\ Yield)}{(Theoretical\ Yield)} \times 100$$

Drug permeation study by Franz diffusion

A Keshary-Chien (K-C) type diffusion cell was used to evaluate drug permeation through a transdermal barrier. The cell consisted of a donor compartment (with the drug) and a receptor compartment (with receptor solution), separated by a barrier. The system included a temperature-controlled jacket and a sample port. Heat was applied using a hot plate, and the receptor solution was stirred with a magnetic stirrer using a star-head magnet.[26]

Antibacterial and antifungal activity of Organogel formulation

Antibacterial Activity

MHA plates were inoculated with *Staphylococcus aureus*. Sterile discs (6 mm) were loaded with 100 µL of *Allium sativum* organogel (1000 µg/mL) and placed on the agar. A marketed gel served as the positive control, and sterile water or plain MHA as the negative control. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured to assess antibacterial activity.[27]

Antifungal activity

SDA plates were inoculated with *Candida albicans*. Sterile discs (6 mm) containing 100 µL of *Allium sativum* organogel (1000 µg/mL) were placed on the agar. Marketed gel and sterile water/plain SDA served as positive and negative controls, respectively. Plates were incubated at 37 °C for 24–48 hours, and zones of inhibition were measured to assess antifungal activity.[27]

Stability study

The optimized gel formulation was kept at room temperature for one month, and after one month the evaluation parameters were checked. The evaluation parameters such as pH, spreadability, drug content, and viscosity were performed.[27]

RESULTS AND DISCUSSION

PREFORMULATION STUDY

Preliminary characterization of *Allium sativum* extract

Organoleptic Properties

The organoleptic characteristics of *Allium sativum* extract, including colour, odour, and appearance, were evaluated. The extract exhibited a brown colour, characteristic garlic odour, and fine powder appearance. These findings were consistent with reported literature values, confirming the identity and quality of the extract.

Table. 5 organoleptic Properties of Extract

Identification Test	Observed Result	Reported Result
Appearance	Brown Fine Powder	Brown powder
Colour	Brownish	Brown
Odour	Characteristic	Characteristic
Taste	Characteristic	Characteristic

Solubility of Extract

The extract exhibited maximum solubility in phosphate buffer pH 7.4 (104.89 mg/mL) and ethanol (104.38 mg/mL), indicating its suitability for topical formulation and in vitro diffusion studies. Moderate solubility was observed in water, methanol, and acetonitrile, while only slight solubility was observed in chloroform. Adequate solubility ensures uniform drug distribution within the organogel.

Table. 6 Solubility of *Allium sativum* Extract in Different Solvent

Solvent	Result	Solubility standard range (mg/ml)	Observed value (mg/ml)
Water	Soluble	33-100	39.16
Ethanol	Freely Soluble	100-1000	104.38
Acetonitrile	Soluble	33-100	37.08
Methanol	Soluble	33-100	49
Phosphate Buffer pH 7.4	Freely soluble	100-1000	104.89
Chloroform	Slightly soluble	1-10	3.5

Moisture Content

The moisture content of *Allium sativum* extract was found to be 4.8 %, which falls within the reported acceptable range of > 5%.

Table.7 Moisture Content

Result	Observed value	Reported value
Moisture content	4.8%	> 5%

Ash Content

The total ash content of the extract was found to be 3.75%, which falls within the reported acceptable range of 3–8%. Ash values indicate the amount of inorganic matter present in the plant material. The obtained value suggests minimal contamination with extraneous materials such as soil, sand, or adulterants, thereby confirming the purity and quality of the crude drug.

Table. 8 Ash Content

Result	Observed value	Reported value
Ash content	3.75 %	3-8 %

Acid insoluble ash

The acid-insoluble ash value was determined as 0.97%, which is below the acceptable limit of 1%. Acid-insoluble ash represents the silica and siliceous impurities present in the sample. The low value indicates negligible contamination with earthy materials, confirming the purity of the Allium sativum extract.

Table. 9 Acid insoluble ash

Result	Observed value	Reported value
Acid insoluble ash	0.97%	< 1%

Water soluble ash

The water-soluble ash value was found to be 2.5%, which lies within the reported range of 2–5%. This result reflects the presence of water-soluble inorganic salts naturally occurring in garlic. The obtained value confirms the authenticity of the plant material and supporting the identity and quality of the extract.

Table. 10 Water soluble ash

Result	Observed value	Reported value
Water soluble ash	2.5%	2-5 %

Phytochemical Test

Preliminary phytochemical screening confirmed the presence of tannins, terpenoids, flavonoids, alkaloids, proteins, carbohydrates, and steroids, while cardiac glycosides were absent. These phytoconstituents are known to possess antimicrobial, antioxidant, and anti-inflammatory activities. The presence of sulfur-containing compounds, flavonoids, and terpenoids may contribute significantly to the observed antibacterial and antifungal effects of the extract. The results support the therapeutic potential of *Allium sativum* for topical antimicrobial applications.

Table. 11 Phytochemical Test

Phytochemical compound	Presence
Tannins	+
Terpenoids	+
Flavonoids	+
Cardiac Glycoside	-
Alkaloid	+
Protein	+
Carbohydrate	+
Steroids	+

UV-Vis Spectroscopy

The UV- Spectrum was recorded in the range 200-400 nm. The wavelength of maximum absorbance (λmax) was determined from the scan and the further preparation of standard curve was carried out at the wavelength of maximum absorbance (λmax). The UV spectrum of *Allium sativum* Extract in phosphate buffer pH 7.4 and exhibited wavelength of absorbance maximum at 240 nm respectively**.**

Determination of absorbance maximum (λ max)

The UV spectrum of *Allium sativum* extract exhibited a maximum absorbance (λmax) at 239.8 nm, which closely matched the reported value of 240 nm. The absorption peak may be attributed to sulfur-containing compounds such as allicin and related organosulfur constituents. The comparison between

observed and reported values confirms the identity of the extract and validates the selected wavelength for further analysis.

Table. 12 Wavelength of Maximum Absorbance in Phosphate Buffer pH 7.4

Solvents	Observed λmax (nm)	Standard λmax (nm)	Absorbance
Phosphate buffer pH 7.4	239.8	240	2.3800

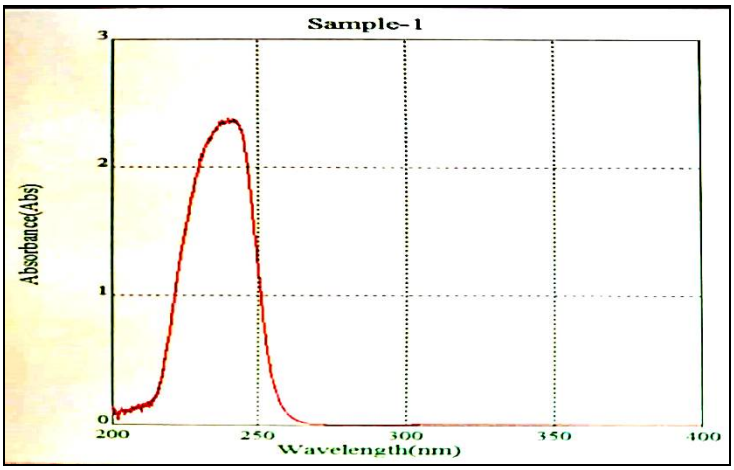

Fig. 2 UV Spectrum of *Allium sativum* Extract in Phosphate Buffer pH 7.4

Construction of calibration curve of *Allium sativum* extract in phosphate buffer pH 7.4

The calibration curve constructed in phosphate buffer pH 7.4 demonstrated a linear relationship between concentration and absorbance over the tested concentration range. The linearity confirmed compliance with Beer-Lambert's law and enabled accurate quantification of the extract during drug content and diffusion studies. The obtained regression equation and correlation coefficient indicated excellent analytical reliability for routine analysis.

The calibration curve exhibited excellent linearity over the concentration range of 10–50 µg/mL with a regression equation of y = 0.0195x + 0.022 and correlation coefficient (R^2) of approximately 0.998, indicating high analytical accuracy and reliability.

Table. 13 Concentration and Absorbance of *Allium sativum* Extract

Concentration (µg/ml)	Absorbance (nm)
10	0.2301
20	0.3987
30	0.5975
40	0.8101
50	0.9909

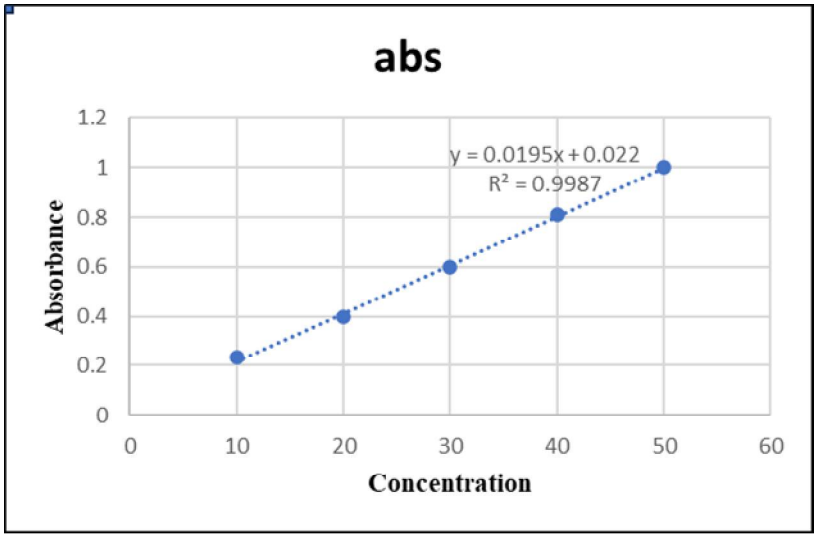

Fig. 3 Calibration Curve of *Allium sativum* Extract in Phosphate Buffer pH 7.4

FTIR Study

FTIR analysis revealed characteristic absorption bands corresponding to O–H, N–H, C–H, C=O, C=C, C–O, and S=O functional groups. The presence of peaks associated with sulfoxide and sulfur-containing compounds confirms the presence of allicin and related organosulfur constituents. These functional groups are responsible for the antibacterial and antifungal activities of garlic extract. The FTIR profile correlated well with reported literature values, confirming the chemical integrity of the extract.

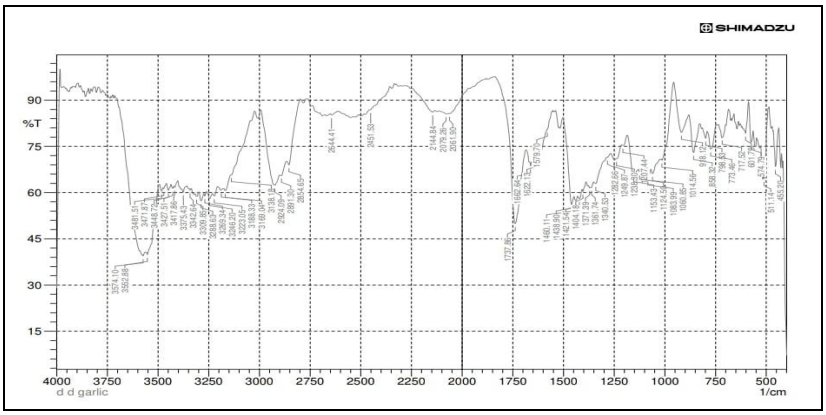

Fig. 4 FTIR Spectrum of *Allium sativum* Extract

Table. 14 FTIR data of *Allium sativum* Extract

Standard Wavelength (cm-1)	Observed Wavelength (cm-1)	Functional group	Interpretation
3200–3600 (broad)	3574, 3552, 3481, 3471, 3448, 3427, 3375, 3342, 3309, 3269, 3223	O–H / N–H stretching	Indicates presence of alcohols, phenols, and amines; related to antioxidant and antimicrobial activity
2850–2950	2854, 2891, 2924, 3138	C–H stretching (aliphatic)	Shows presence of aliphatic hydrocarbon chains; commonly found in essential oils.
2100–2300 (weak)	2061, 2079, 2144, 2451, 2644	C≡C / C≡N stretch (if present)	Presence of alkynes or nitriles, possibly from nitrogenous sulfur compounds.
1700–1750	1737	C=O stretching (aldehydes, esters)	Suggests aldehydes and esters — common in volatile sulfur compounds and flavor agents.
1600–1700	1460, 1579, 1622	C=C / C=O stretch	Indicates alkenes or aromatic rings; associated with flavonoids or polyphenols.
1350–1470	1361,1371, 1404, 1421, 1438,	CH_2 / CH_3 bending	Confirms presence of methyl and methylene groups — often found in fatty acids.
1200–1350	1207, 1238, 1249, 1282, 1340	C–O–C (ethers), C–N	Suggests ethers and amines — typical in organosulfur or amino-containing compounds.
1000–1300	1014, 1060, 1083, 1124, 1153	C–O stretch / S=O (sulfoxide)	Confirms presence of sulfoxides like allicin — key antimicrobial sulfur compound.
650–950	717, 773, 798, 858, 918	C–H bending (alkyl) / C–S	Shows sulfur linkage in alkyl chains — indicates diallyl sulfide or similar structures
500–700	455, 511, 574, 601	C–S stretching (organosulfur)	Strong indication of organosulfur compounds

Drug-Excipient Compatibility

The FTIR spectrum of the extract-excipient mixture showed all major characteristic peaks of the garlic extract without significant shifting, disappearance, or appearance of new peaks. This observation suggests the absence of chemical interaction between the extract and formulation excipients such as

lecithin, Tween 80, oleic acid, and triethanolamine. Therefore, the selected excipients were considered compatible and suitable for organogel formulation.

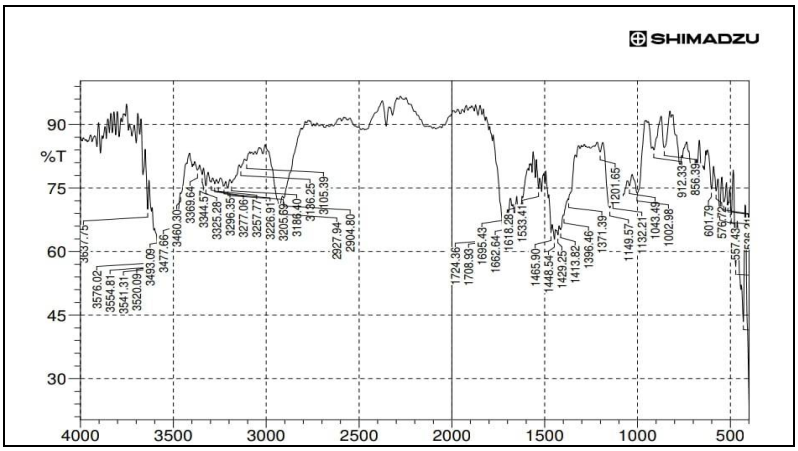

Fig. 5 FTIR Spectrum of *Allium sativum* Extract and Excipients of Organogel

Table. 15 FTIR Data of *Allium sativum* Extract and Excipients

Standard Wavelength (cm-1)	Observed Wavelength (nm)	Functional group	Interpretation
3200–3600 (broad)	3576, 3541, 3520, 3460, 3360, 3328, 3266	O–H / N–H broad stretching – indicates hydrogen bonding between garlic phytochemicals and lecithin	Confirms presence of alcohols, phenols, and amines; indicates strong hydrogen bonding interactions
2850–2950	2927, 2904	C–H stretching (aliphatic) – confirms presence of aliphatic chains from garlic oils and lecithin	Suggests the presence of long-chain fatty acids or hydrocarbon backbones typical of oils and phospholipids
2100–2300 (if present)	-	C=O stretching (carbonyl) – from lecithin esters and garlic aldehydes	Indicates carbonyl groups from esters or aldehydes in garlic or excipient matrix
1700–1750	1724, 1708	C=C stretching or amide regions – possible unsaturated fatty acids or protein traces	Presence of unsaturated lipid components or minor protein residues
1600–1700	1662, 1633	CH_2 / CH_3 bending – typical for lipid components	Suggests hydrocarbon chains and supports the presence of lecithin and garlic lipids
1350–1470	1465, 1449, 1438, 1371	P=O or C–N stretching – confirms presence of phospholipids (lecithin)	Indicates lecithin's phospholipid nature due to phosphate and nitrogenous bonds
1200–1350	1386, 1201, 1149, 1102	C–O stretching – seen in alcohols, esters, and ethers in both garlic and lecithin	Confirms presence of ester or ether linkages, common in garlic compounds and emulsifiers
1000–1300	1002	S=O or C–H bending (out-of-plane) – sulfur-containing compounds of garlic	Supports presence of sulfoxides like allicin and sulfur-containing active principles
650–950	912, 856, 816	Aromatic / sulfur peaks – confirms garlic's organosulfur profile retained	Characteristic of diallyl sulfides and other organosulfur constituents in garlic
500–700	601, 557		Strong evidence of organosulfur bioactives retained from garlic

HPLC (High Performance Liquid Chromatography)

The two chromatograms were analyzed by comparing the *Allium sativum* extract sample with a standard allicin sample to confirm the presence of allicin. HPLC chromatograms of the standard allicin and *Allium sativum* extract showed comparable retention times of 3.448 min and 3.229 min, respectively. The similarity in chromatographic behavior confirms the presence of allicin within the extract responsible for antifungal and antimicrobial activity.

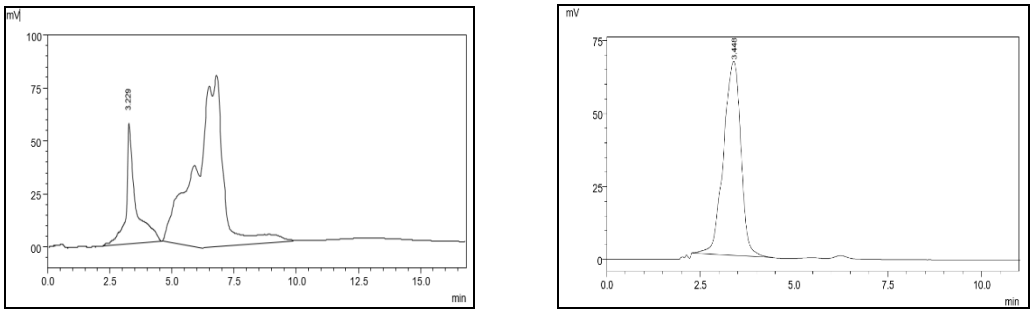

Fig. 6 HPLC Chromatogram of Sample (*Allium sativum* extract) and Standard (Allicin)

Table. 16 HPLC Analysis of Allicin

Parameter	Chromatogram (Standard)	Chromatogram (Sample)	Similarity
Retention Time	3.448 minutes	3.229 minutes	High
Peak Shape	Sharp, well-defined	Sharp, well-defined	High
Peak Height	70mV	59 mV	Medium
Baseline Stability	Stable	Stable	High
Units of Measurement	mV	mV	High
Chromatographic Conditions	Same	Same	High

MIC of *Allium sativum* extract

The extract exhibited a minimum inhibitory concentration of 1.5 mg/mL against both *Staphylococcus aureus* and *Candida albicans*. At this concentration, clear zones of inhibition measuring 10 mm and 9 mm were observed, respectively. The results confirm the antibacterial and antifungal activity of the extract.

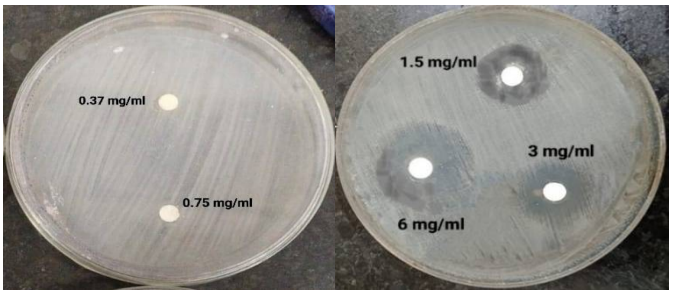

Fig. 7 Antibacterial Activity

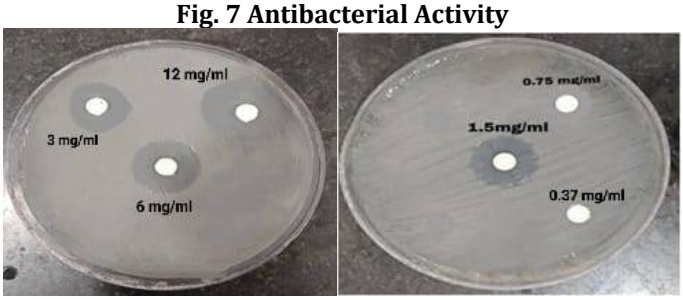

Fig. 8 Antifungal Activity

Table. 17 Minimum Inhibitory Concentration of *Allium sativum* Extract

Sr. No.	Species	MIC	Zone Of Inhibition
1.	*Staphylococcus aureus*	1.5mg/ml	10 mm
2.	*Candida albicans*	1.5mg/ml	9 mm

OPTIMIZATION STUDY

Box-Behnken design was employed for further study. Viscosity and spreadability were performed and the results were fed into the design expert software. The data was analyzed by ANOVA and was reported to be significant.

Table. 18 Results of optimization study using Box-Behnken design

	Factor 1	Factor 2	Factor 3	Response 1	Response 2
Batches	A: Gelling agent (Soya lecithin)	B: Surfactant (Tween 80)	C: pH adjuster (Triethanolamine)	Viscosity	Spreadability
	%	%	%	Cp	gm.cm/sec
F1	2.5	7.5	0.5	2990	22.8
F2	10	6.25	0.4	4500	20.4
F3	6.25	6.25	0.5	3450	22.2
F4	6.25	6.25	0.5	3600	21.6
F5	6.25	7.5	0.4	3610	21.3
F6	2.5	5	0.5	3200	24.3
F7	2.5	6.25	0.6	2910	23.01
F8	10	7.5	0.5	4300	20.28
F9	10	5	0.5	4610	20.51
F10	6.25	6.25	0.5	3600	21.8
F11	10	6.25	0.6	4600	20.38
F12	2.5	6.25	0.4	2900	23.8
F13	6.25	5	0.6	3590	22.18
F14	6.25	7.5	0.6	3550	20.8
F15	6.25	6.25	0.5	3600	22.01
F16	6.25	6.25	0.5	3610	22.1
F17	6.25	5	0.4	3500	22.59

Optimization of Box-Behnken design batches

The Box-Behnken design successfully evaluated the effects of soya lecithin, Tween 80, and triethanolamine on viscosity and spreadability. An increase in lecithin concentration significantly increased viscosity due to enhanced three-dimensional network formation within the organogel matrix. Conversely, higher surfactant levels reduced viscosity and improved spreadability. The statistical model was found significant, indicating that the selected variables effectively influenced formulation performance and enabled optimization.

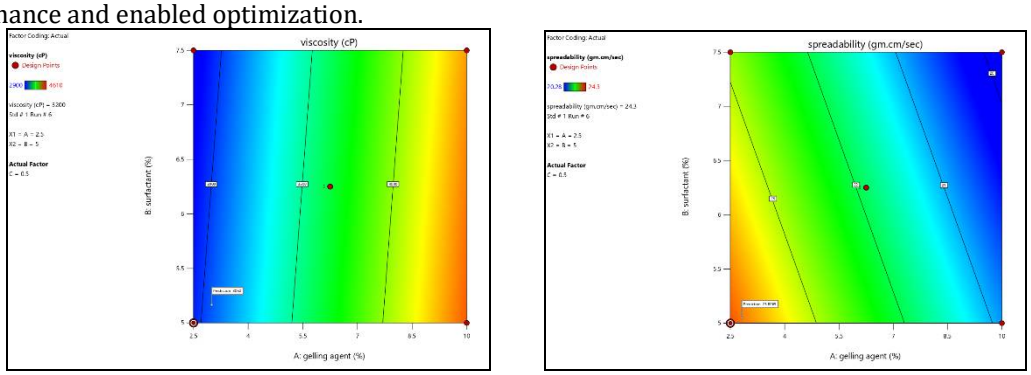

Fig. 9 Contour Plot of responses a.Viscosity, b. Spreadability

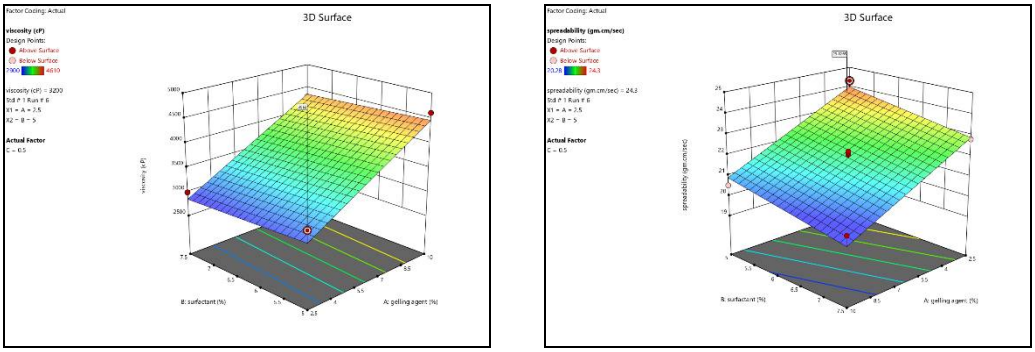

Fig. 10 3D plot of Responses of a. Viscosity, b. Spreadability

FORMULATION AND DEVELOPMENT OF *ALLIUM SATIVUM* EXTRACT ORGANOGEL

A stable organogel was successfully prepared. Lecithin acted as the primary organogelator, forming a three-dimensional network capable of entrapping the extract and oily phase. The prepared formulation

exhibited acceptable consistency, homogeneity, and ease of application. No evidence of phase separation or precipitation was observed, indicating successful gel formation.

Fig. 11 Organogel of *Allium sativum* extract

EVALUATION OF *ALLIUM SATIVUM* ORGANOGEL

All prepared formulations showed satisfactory physicochemical characteristics. The pH ranged from 5.05–6.87, which is compatible with skin physiology and minimises irritation risk. Viscosity ranged between 2900–4610 cP, indicating adequate consistency for topical application. Spreadability values ranged from 20.28–24.30 g.cm/sec, suggesting ease of application. Among all formulations, batch F6 exhibited the most desirable balance of viscosity (3200 cP), spreadability (24.3 g.cm/sec), and drug content (91.28%), and was therefore selected as the optimized formulation.

Physical appearance

Table. 19 Organoleptic properties

Sr. No.	Test	Observation
1	Colour	Dark brown
2	Odour	Characteristics
3	Appearance	Smooth
4	Washability	Easily washable

Table. 20 Evaluation of Organogel

Sr. No	Batch	pH	Viscosity (cP)	Spreadability (gm.cm/sec)	% Drug Content
1	F1	6.35	2990	22.8	82.05
2	F2	5.75	4500	20.4	78.29
3	F3	6.37	3450	22.2	87.17
4	F4	5.05	3600	21.6	88.54
5	F5	6.56	3610	21.3	89.91
6	F6	6.25	3200	24.3	91.28
7	F7	6.82	2910	23.01	87.86
8	F8	6.71	4300	20.28	89.57
9	F9	6.87	4610	20.51	90.59
10	F10	5.06	3600	21.8	89.57
11	F11	6.54	4600	20.38	81.90
12	F12	6.23	2900	23.8	88.97
13	F13	6.34	3590	22.18	89.88
14	F14	5.35	3550	20.8	82.05
15	F15	6.76	3600	22.01	78.40
16	F16	6.23	3610	22.1	87.17
17	F17	5.60	3500	22.59	88.54
18	MARKETED GEL (MINYM GEL)	6.63	4109	23.4	94.21
19	MARKETED GEL (CANDID GEL)	6.80	3980	23.9	95.44

Drug Permeation Study by Franz Diffusion Kinetics of Drug Release

The drug release study was exclusively carried out for the optimized gel formulation. The obtained release profile was fitted into various kinetic models such as Zero order, First order, Higuchi, and

Korsmeyer-Peppas. The results indicated that the drug release followed Korsmeyer-Peppas model with a diffusion-controlled mechanism. The kinetic data are presented in Table. 21.

Table. 21 Kinetics of Drug Release

Cumulative % Drug Release			
Time (Min)	**F6**	**MARKETED GEL (MINYM GEL)**	**MARKETED GEL (CANDID GEL)**
15	1.70	2.48	3.67
30	4.47	6.25	8.86
45	8.75	10.59	13.67
60	13.88	16.03	19.24
90	19.35	22.62	26.70
120	26.18	30.29	34.54
150	33.70	38.81	43.14
180	41.91	48.07	52.5
210	51.14	57.92	62.61
240	62.08	68.62	73.10
270	74.39	79.81	84.35
300	87.04	91.92	95.98

The above table presents the cumulative percentage of drug release over time for different formulations: F6 (optimized batch), Minym gel and Candid gel. The data indicates that at the 15-minute mark, the drug release ranges from 1.70% to 3.67%, gradually increasing over time. By 300 minutes, the release percentages reach their peak, with Minym gel showing the highest release at 91.92%, followed closely by Candid gel at 95.98%. The optimized formulation F6 also demonstrates significant release, reaching 87.04% at the end of the study. This trend illustrates the varying efficacy of different formulations in releasing the drug over a five-hour period.

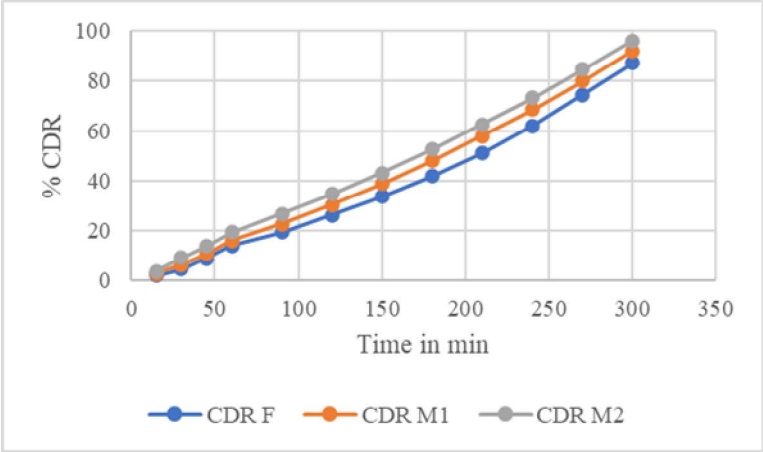

Fig. 12 Comparative Drug Release Study of optimized batch F6 and Marketed Gel

The graph illustrates the cumulative percentage of drug release over time for different formulations F6 (optimized batch), Minym gel and Candid gel. Each formulation's release profile is tracked from 15 to 300 minutes. The data shows a general upward trend, indicating increasing drug release over time for all formulations. Candid gel shows the highest release throughout the study, closely followed by Minym gel, which also exhibits substantial drug release. The optimized batch F6 demonstrates a steady and controlled release profile, reaching nearly 87.04% by 300 minutes. The graph highlights the difference in drug release rates and supports the potential of F6 as a competitive alternative to marketed gels.

Drug release kinetics of optimized batch F6

The optimized formulation F6 demonstrated sustained drug release over 300 minutes, achieving a cumulative release of 87.04%. Release data were fitted to various kinetic models, including Zero-order, First-order, Higuchi, and Korsmeyer-Peppas models. The highest correlation coefficient was obtained with the Korsmeyer-Peppas model, indicating diffusion-controlled drug release. The organogel matrix

likely acted as a diffusion barrier, enabling prolonged release of active constituents and enhancing therapeutic efficacy.

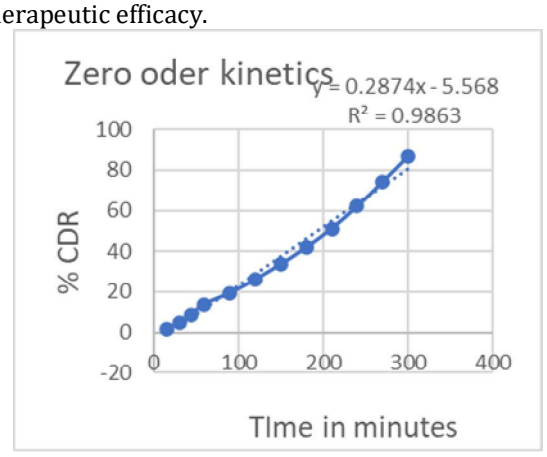

Fig. 13 Zero Order Drug Release Kinetics

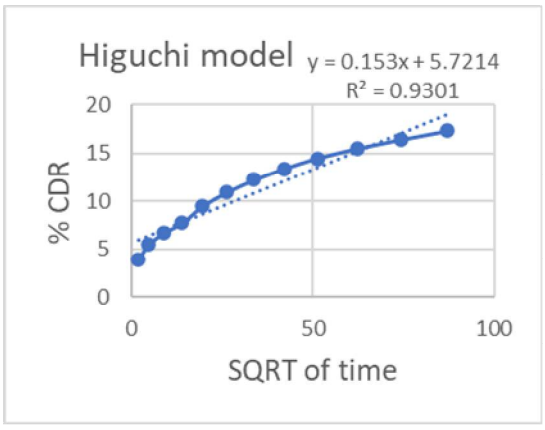

Fig. 14 Higuchi Model Drug Release Kinetics

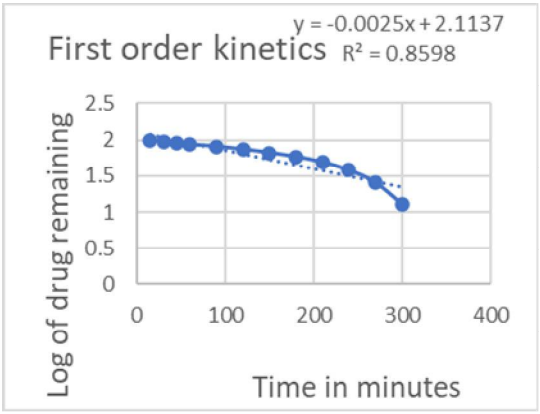

Fig. 15 First Order Drug Release Kinetics

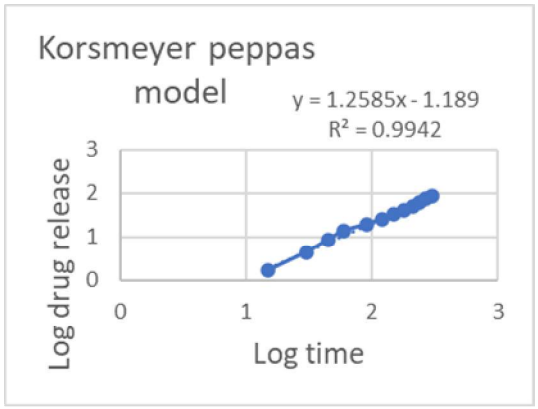

Fig. 16 Korsmeyer-Peppas Drug Release Kinetics

Antibacterial and Antifungal Activity of Organogel

Antibacterial activity

The formulated organogel exhibited significant antibacterial activity against *Staphylococcus aureus*, producing a 12 mm zone of inhibition. Although slightly lower than the marketed gel (15 mm), complete inhibition of bacterial growth was observed. These findings demonstrate the suitability of the developed organogel as a natural antibacterial topical formulation.

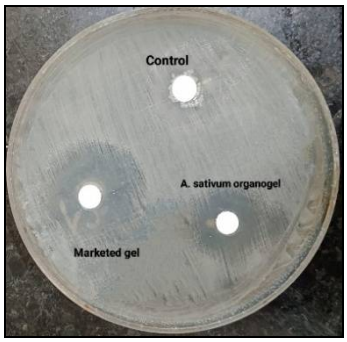

Fig. 17 Antibacterial Activity, A: Formulation, B: Marketed Gel (Positive Control), C: Plain Mueller-Hinton Agar (Negative Control)

Table. 22 Antibacterial Activity of Organogel

Gel formulations	Species	Zone of inhibition (mm)	Result
Marketed gel	*Staphylococcus aureus*	15 mm	No growth
Allium sativum Organogel	*Staphylococcus aureus*	12 mm	No growth

Antifungal activity

The organogel produced an 8 mm zone of inhibition against *Candida albicans*, while the marketed gel showed a 10 mm zone. The observed antifungal activity confirms the retention of garlic bioactivity within the organogel matrix. The results support the potential application of the formulation in fungal skin infections.

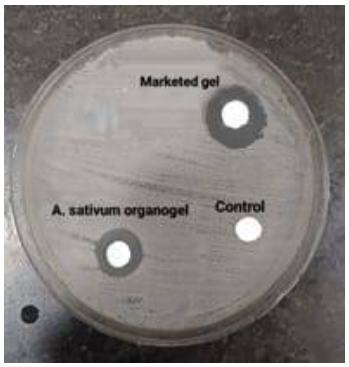

Fig. 18 Antifungal Activity A: Formulation, B: Marketed Gel (Positive Control), C: Plain Sabouraud Dextrose Agar (Negative Control)

Table. 23 Antifungal Activity of Organogel

Gel formulations	Species	Zone of inhibition (mm)	Result
Marketed gel	*Candida albicans*	10	No growth
Allium sativum Organogel	*Candida albicans*	8	No growth

Stability studies

The evaluation study of F6 batch was conducted one month later. No significant changes were observed in pH, viscosity, spreadability, or drug content and all results have proven satisfactory.

Table. 24 Stability Study Results

Evaluation Tests	Initial result	After one-month Results
pH	6.25	6.25
Viscosity	3200	3205
Spreadability	24.3	24.1
Drug content	91.28	91.23

CONCLUSION

The present research successfully demonstrated the formulation and evaluation of an Allium sativum (garlic) extract-loaded organogel as a novel topical drug delivery system with significant antibacterial and antifungal potential. The optimized organogel (F6), developed using a Box-Behnken design, showed excellent physicochemical characteristics such as appropriate viscosity, pH, spreadability, and high drug content. FTIR and HPLC analyses confirmed the presence and stability of bioactive constituents, particularly allicin. The organogel exhibited effective antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*, with sustained drug release following Korsmeyer-Peppas kinetics, indicating diffusion-controlled behavior. Stability studies confirmed the robustness of the formulation over time. Overall, the study validates the use of garlic-based organogels as a promising, biocompatible, and effective approach for treating bacterial and fungal skin infections.

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CONFLICT OF INTEREST

Author declares that they have no conflict of interest.

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