ORIGINAL ARTICLE

RB SCIENCE
ENTERPRISING BIOSCIENCE RESEARCH

JOURNAL OF PHARMACOLOGY AND BIOMEDICINE

Published by RB Science Home Page: www.jpbiomed.com

Formulation And Evaluation Of Allicin Gel And Determination Of Anti-bacterial Activity

Afreen Sultana*, Hajira Rahman, Mareyam Maliha, Nazifa Siddiqua and Tamkenat Noor

Department of Pharmaceutics, RBVRR Women's College of Pharmacy, Barkatpura, Hyderabad - 500027, Telangana, India

Article History

Received on: 10/10/2024

Revised on: 19/12/2024

Accepted on: 19/01/2025

Published on: 10/04/2025

Keywords

Allicin

Antibacterial property

Carbopol 934

Gram positive bacteria

Ultra-Centrifugation

Homogenization

ABSTRACT

The main aim of the study was to formulate, optimize, evaluate topical preparation of Allicin gel and determine antibacterial properties. Allicin is an organosulfur compound used in the treatment of bacterial skin infections. Total six formulations (F1, F2, F3, F4, F5 & F6) were formulated by using different concentration of allicin with carbopol 934 and determine antibacterial property against gram positive bacterias - Staphylococcus Aureus, Bacillus Cereus, Streptococcus Mutans and Streptococcus Gordonii. The results reveal that the study was within acceptable limits as per the ICH guidelines. Formulated gels were homogenized and stable. Topical application of the herbal gel F6 containing 0.6 mg conc of allicin showed better release (78.2%) with significant antibacterial properties against three bacteria's i,e. Bacillus Cereus, Streptococcus Mutans and Streptococcus Gordonii.

*Corresponding Author

Afreen Sultana

Email: afreensultana704@gmail.com

JOURNAL OF PHARMACOLOGY AND BIOMEDICINE

ISSN No. 2456-8244

Publication Hosted by jpbiomed.com

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Introduction

Herbal gels are topical preparations intended to deliver plant-derived therapeutic agents directly to the skin. They offer numerous advantages, such as easy application, targeted treatment, and typically fewer side effects compared to systemic medications. Allicin is the main component of garlic (Allium Sativum), which exhibits wide range of antimicrobial including bactericidal, antifungal, antiparasitic, and antiviral activities against a wide range of Gram-negative and Gram-positive bacteria (A.Y Uchida et.al) . Allicin is formulated in gel for topical application that may account as an option for prophylaxis against various bacterial infections. Various bacterial species are responsible for effecting lethal skin diseases such as impetigo, Folliculitis, Skin Abscess etc and are caused through certain bacteria such as Staphylococcus Aureus, Bacillus Cereus, Streptococcus Mutans, Streptococcus Gordonii etc. Allicin acts as potent antibacterial activity, which has leading activity against various bacteria's through, Inhibition of Sulfhydryl-Dependent Enzymes: Allicin inhibits sulfhydryl-dependent enzymes, including alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase. By disrupting these essential enzymes, it interferes with bacterial metabolism and growth. Another way is through Thiol-Containing Proteins: Allicin selectively targets thiol-containing proteins and enzymes in microorganisms.

This disruption affects their vital metabolic pathways, leading to antibacterial effects and whereas, S-thio allylation. Allicin achieves its strong antimicrobial activity against bacteria and fungi by S-thiol alkylating protein thiols and low molecular weight thiols.(B.Kirstin Mösbauer, et.al 2021). Gels are getting more popular nowadays because they are more stable and can provide controlled release than other semisolid preparations like creams, ointments, pastes, etc. Gel formulation can provide better absorption characteristics and hence increase the bioavailability of the drug .Few properties of gel are : Ideally the gelling agent must be inert, safe and cannot react with another formulation constituents , the gelling agent should produce a sensible solid like nature at the time of storage which is easily broken when exposed to shear forces produced by squeezing the tube, It should have suitable antibacterial agent, The topical gel must not be sticky, Each component is continuous throughout the system.(C.loyd VA.,et.al 2011)(D-ofnerCM., et.al 2007) .

Garlic is an excellent alternative to the antibiotics used currently. Extract from the underground garlic bulb, especially its compound allicin (diallyl-thiosulfinate), inhibits the growth of many species of Gram-positive and Gram-negative bacteria. (E.Yoshida et.al.1999; Tsao and Yin 2001; Bagri and Douglas2005) .

Methods and materials

Materials

All the substances and ingredients such as : Carbopol 934, demineralized water, disodium edetate, triethanolamine, and propylene glycol solution was obtained from RBVRR Women's college of Pharmacy, Barkatpura, Hyderabad.

Methods

Extraction Procedure of Allicin :

Fresh Garlic Clove samples were peeled and crushed using hand-held press and kept for 5min at 4°C in beaker. Approximately 1g of crushed sample was accurately weighed and

transferred to 50 ml centrifuged tube, Prior to adding one of seven homogenization solution 10 ml to each tube. Tubes with slurry were then placed in an ice bath (0-4°) and the samples were homogenized for 1 min using a Heidolph Silent Crusher M homogeniser. 30ml of extraction solution was added to tubes then shaken for 10 min at room temperature and then centrifuged at 5820 x g for 10 min at 4°C. The supernatant was collected, and the residual pellet was re-extracted with a further 10 ml of extraction solution. The two Supernatants were combined and filtered through a 0.22µm hydrophilic PTFE syringe filter and analysis and extract is stored up to 24 h at 4°c (B.T. Nguyen et. al, 2021) **.**

Formulation of Allicin Gel

Preparation of gel base

Carbopol 934 was dissolved slowly with stirring in 60 mL of demineralized water for 1 h to avoid agglomeration. Then disodium edetate and triethanolamine were dissolved in 10 mL of demineralized water separately and stirred for 10 min. Mixed 4.83 mL of propylene glycol in 12 mL of demineralized water while stirring for 10 min. Disodium edetate and triethanolamine solution were added to Carbopol solution and the pH was then adjusted to 7.4 by stirring the solution for 10 min. Then a propylene glycol solution was added with stirring for 10 min until a clear consistent gel base was obtained.

Preparation of gel formation :

Six topical gel formulations were prepared using allicin extract as per drug formulation manual where F1 to F6 formulations were made using the gel base of Carbopol 934 (1.5 %). Details of formulation composition are recorded in the below table. (H.Nandgude et al., 2008).

Evaluation of Allicin gel

UV- visible Spectrophotometric Study

Preparation of Phosphate buffer pH-7.4: Place 250 ml of 0.2 M Potassium di-hydrogen phosphate, add 393.4ml of 0.2 M sodium hydroxide and then add distilled water to make up the volume 1000ml.

Preparation of 0.2 M Potassium di-hydrogen phosphate solution: Dissolve 27.218g of potassium di-hydrogen phosphate in sufficient distilled water containing in the 1000 ml volumetric flask and to make up to the volume 1000ml.

Preparation of 0.2 M sodium hydroxide: Solution of 0.2 Molar may be prepared by dissolving 8 gms of sodium hydroxide in sufficient distilled water contained in the 1000 ml volumetric flask and make up to the volume 1000ml.

Preparation of solutions for Calibration curve

Stock solution: 5.055 ml of allicin extraction is diluted to 10 ml with phosphate buffer pH 7.4 to get a stock solution containing 100 µg/ml of drug. The stock solution was filtered through Whatman filter paper No.41.

Dilutions: Take the respective samples (0.2ml,0.4ml, 0.6ml, 0.8ml, 1ml) in each test tube, add phosphate buffer 7.4 to make total volume of 10 ml to produce (2,4,6, 8, 10µg/ml) respectively.

Determination of absorption maxima:

A UV absorption maximum was determined by scanning 10µg/ml solution, in between 200-400 nm by using UV-visible spectrophotometer.

Table - 1: Formulation Development

Formu-lation	Allicin extract (mg)	Carbopol-934 (g)	Triethano-lamine (ml)	Disodium EDTA (g)	Propylene glycol (g)	Distill H_2O
F1	1	1.5	1.5	0.005	5	Q.S
F2	2	1.5	1.5	0.005	5	Q.S
F3	3	1.5	1.5	0.005	5	Q.S
F4	4	1.5	1.5	0.005	5	Q.S
F5	5	1.5	1.5	0.005	5	Q.S
F6	6	1.5	1.5	0.005	5	Q.S

Preparation of Calibration curve:

The standard solutions for the extract having concentration 2, 4, 6, 8, 10 µg/ml were prepared with phosphate buffer pH 7.4 from the stock solution. The absorbance of solutions of pure extract were measured at 237 λ max and a calibration curve was plotted between absorbance v/s concentration to get the linearity and regression equation.

Linearity and calibration:

Linearity of the calibration curve was determined by plotting absorbance (nm) (on y-axis) versus concentration (µg/ml) (on x-axis) of allicin extract. Prepare a calibration curve by measuring the absorbance at 237 nm. Determined slope (m), intercept (y), regression coefficient (R_2) by statistical analysis.

Estimation of active constituents in gel formulation (net content) :

Each formulation (0.5 g) was taken in a 25 mL volumetric flask and made up to volume with methanol and shaken well to dissolve the active constituents in methanol. The solution was filtered through Whatman filter paper and 0.1 mL of the filtrate was pipetted out 1 ml and diluted to 10 mL with methanol. The content of active constituents was estimated spectrophotometrically by using standard curves plotted at 237 nm (λmax of active constituents in the extracts) (Nandgude et al., 2008).

Figure- 1: 6 - Volumetric flask Gel Formulation.

Extrudability

A closed collapsible tube containing about 20 g of gel was pressed firmly at the crimped end and a clamp was applied to prevent any roll back. The cap was removed and the gel was extruded. The amount of the extruded gel was collected and weighed. The percentage of the extruded gel was calculated (I.Nappinai, Pakalapati, Arimilli, 2006).

pH measurement

pH measurement of the gel was carried out using a digital pH meter by dipping the glass electrode completely into the gel system to cover the electrode. (J.Queiroz et al., 2000)

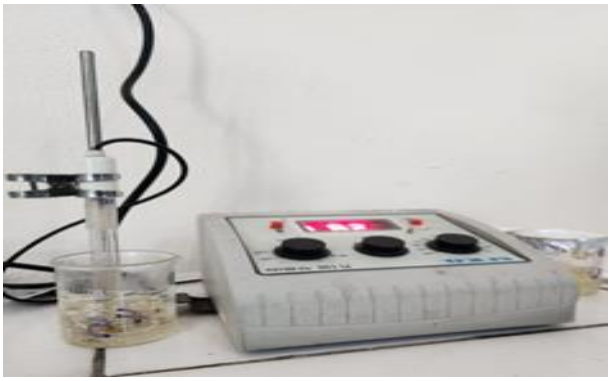


Figure - 2 : PH Meter.

Appearance and Homogeneity

Physical appearance and homogeneity of the prepared gels were evaluated by visual perception

Viscosity

Viscosity of gel was determined using Brookfield viscometer at 25°C with a spindle speed of the viscometer rotated at 100 rpm (K.Nayak et al., 2005).

Figure - 3 : Brookfield viscometer

Spreadability :

Two sets of glass slides of standard dimensions were taken. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slides then the two slides was pressed uniformly to form a thin layer. A 1g weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated for three times and the mean time was taken for calculation. (Jain et al., 2007). Spreadability was calculated by using the following formula:

$$S = m \times l/t$$

where, S= spreadability, m-weight tied to upper slides (1 g), l- length of the glass slide (7.5 cm), t- time taken in sec.

***In vitro* diffusion profile:**

In vitro diffusion studies for all formulations were carried out using Franz diffusion cells. The diffusion cell apparatus was fabricated locally as an open-ended cylindrical tube with 3.7994 cm2 area and 100 mm height having a

diffusion area of 3.8 cm^2. Phosphate buffer (pH 7.4) was used as receptor media. Diffusion membrane was used as dialysis membrane. The dialysis membrane was tied to the diffusion cell (donor cell) such that the dialysis membrane was in intimate contact with the release surface of the formulation in the donor cell. Phosphate buffer solution, pH 7.4 (100 mL) was added to a donor compartment prior to be mounted on the diffusion cell. A weight of 1 g of gel was taken on to the dialysis membrane and was immersed slightly in 100 mL of receptor medium, which was continuously stirred. The entire system was maintained at 37±1 ºC. An aliquot of 5 mL was withdrawn at specific time intervals up to 6 h, and was estimated spectrophotometrically at 237 nm. After each withdrawal, the diffusion medium was replaced with an equal volume of fresh diffusion medium. The cumulative percent release was calculated for each time (in h) interval. (Martin, 1994).

Figure - 4: *In vitro* diffusion profile of topical herbal gels.

Agar well diffusion method

The agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. After the inoculum got solidified. Then, a hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the antibacterial agent i,e allicin gel formulation at different concentrations (0.1mg, 0.2mg, 0.3mg, 0.4mg, 0.5mg and 0.6mg) is introduced into the well. Then, agar plates are incubated under an incubator with 37℃. The antibacterial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested within 24 hours. Evaluate the zone of inhibition in mm after 24 hours of diffusion and compare the results with standard i,e. Tetracycline.

Results and Discussions

Extraction of Allicin

Figure - 5: Homogenizer

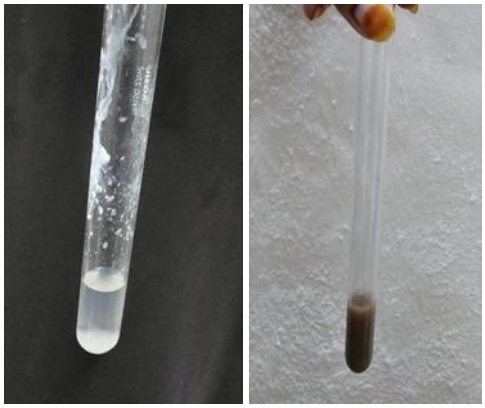


Figure - 6: Qualitative Test (A) Sediment (B) Supernatant

Formulation of Allicin Gel

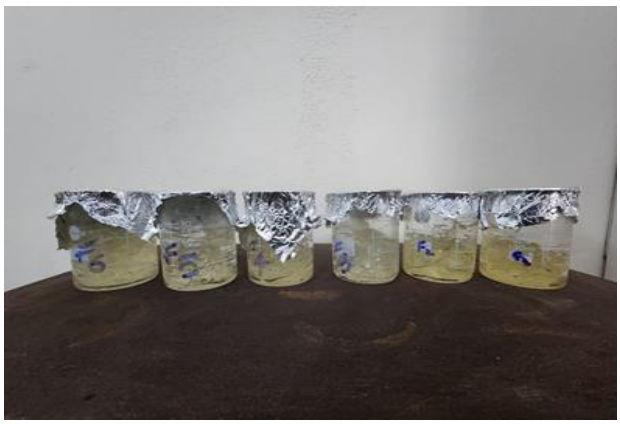
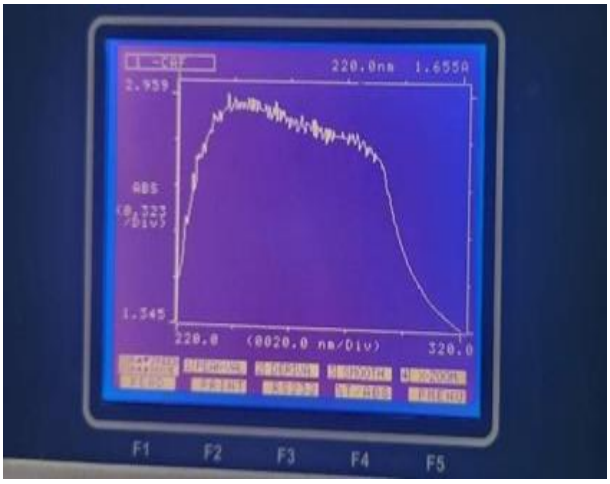


Figure - 8: Formulations (F1, F2, F3, F4, F5 & F6

Evaluation of Allicin Gel :

Calibration curve of Allicin Extract :

The standard graph was found to be linear with R2 value of (0.9939) as shown in figure. The method obeyed beer Lambert laws in the concentration range of 2-10 ug/ml.



Figure—9: Absorption maxima () of Allicin extract

Table –2: Standard plot values

Conc(µg/ml)	Absorbance
2	0.0156
4	0.0248
6	0.0365
8	0.0428
10	0.0568

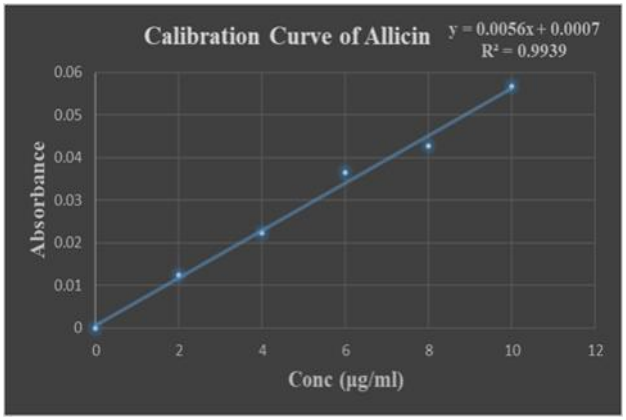


Figure - 10: Calibration curve of allicin extract

The standard graph was found to be linear with R2 value of (0.9939) as shown in figure. The method obeyed beer Lambert laws in the concentration range of 2-10 ug/ml.

Evaluation parameters for all formulations

Six gel formulations F1 to F6 prepared using Carbopol polymers were evaluated for physical appearance, pH, viscosity, spreadability , net content, extrudability and in vitro diffusion profile. Results of the study were within acceptable limits of the ICH guidelines (Table –3).

In vitro diffusion profile of tropical herbal gels

The in vitro release profiles of all the six formulations made using carbopol 934 elicited almost 80 % release from the formulation within 6 h. The in vitro release characteristics of the prepared topical herbal gel formulations were quite encouraging. Among the formulations, F6 showed better release (78.2%) characteristics than F1, F2, F3, F4 and F5 (Table –4) .

Table - 4: In vitro diffusion

Table 3 : **Evaluation parameters for topical herbal gel formulation**

Code		Conc(%)	pH	Viscosity (poise)	Spreadability (g cm/sec)	Net content % w/w	Extrudability	Physical appearance
F1		0.1	7.33	55	7.05	99.7	Good	Light yellow, smooth, homogenous, translucent
F2		0.2	7.27	56	8	105	Excellent	Light yellow, smooth, homogenous, translucent
F3		0.3	7.73	52	9	105	Good	Light yellow, smooth, homogenous, translucent
F4		0.4	7.82	58	9.6	105	Excellent	Light yellow, smooth, homogenous, translucent
F5		0.5	7.92	58	10	101	Excellent	Light yellow, smooth, homogenous, translucent
F6		0.6	7.82	60	10.4	101	Excellent	Light yellow, smooth, homogenous, translucent

tropical herbal gels (F1-F6)

TIME (HOUR)	F1	F2	F3	F4	F5	F6
1	9.88	11.45	17.4	12.15	13.32	19.5
2	16.8	19.68	23.6	26.55	24.08	28.7
3	24.6	26.8	37.2	39.15	33.84	32.5
4	29.9	37.4	48	48.6	41.52	43
5	35.6	49.35	64.2	64.8	52.8	58.8
6	48.6	58.2	73.2	72	60.48	78.2

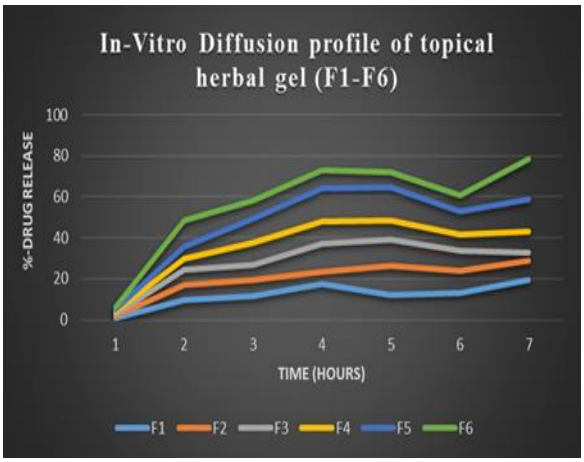


Figure - 12: In vitro diffusion profile of tropical herbal gels (F1-F6)

Determination of antibacterial activity

The antibacterial characteristics of the prepared topical herbal gel formulations were evaluated. Among the formulations, F6 showed better zone of inhibition (BC-2.4 cm, SG-1.4 cm, SM-1.8 cm & SA-Nil) characteristics than F1, F2, F3, F4 and F5 (Table –5).

Table –5: Zone of inhibition of F6 gel

Sr. No	Bacteria	Zone of inhibition of gel F6 (mm)	Zone of inhibition of standard drug (mm)
1.	Bacillus cereus	24	16
2.	Staphylococcus aureus	nil	15
3.	Streptococcus gordonii	25	20
4.	Streptococcus mutans	18	17

Figure - 12: Bacillus cereus

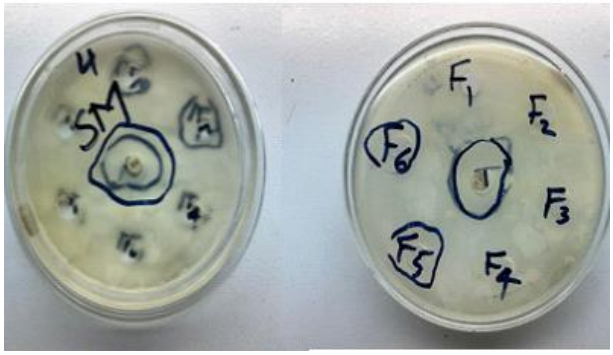


Figure -13: Streptococcus mutans

Figure - 14: Streptococcus gordonii

Figure - 15: Staphylococcus aureus

Conclusion

From the present study, the following conclusion shows that allicin gel is formulated and shows antibacterial activity against various bacteria such as Staphylococcus aureus, Bacillus cereus, Streptococcus mutans, and Streptococcus gordonii, with diverse concentrations of allicin as an antibacterial agent. It has been found to be effective in inhibiting bacterial activity. Allicin gel with 0.6 mg i,e. F6 is found to be optimized and highly preventive against bacteria. In addition, other parameters such as pH measurement, viscosity, spreadability, and diffusion profile were assessed, and the results of the study were within the acceptable limits of the ICH guidelines.

Acknowledgement : Nil

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Cite this article as

Sultana A, Rahman H,Maliha M,Siddiqua N,Tamkenat N. Formulation And Evaluation Of Allicin Gel And Determination Of Antibacterial Activity. J Pharmacol Biomed. 2025; 9(1): 791-801