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Formulation Design and Evaluation of an Antimicrobial Hydrogel Patch Containing Allium Ascalonicum Extract

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ABSTRACT
Skin and tissue infections are extremely difficult to treat and are frequently made worse by antibiotic resistance and side effect from traditional therapies. The creation and assessment of anti-microbial hydrogel patch contain extract from Allium Ascalonicum is the main goal of this work. Antimicrobial qualities of the extract, such as its anti-fungal and anti-bacterial capabilities, are well documented. This work offers a novel method of using a hydrogel patch that is bioactive and contains extract from Allium Ascalonicum. The procedure entails locating shallots, isolating bioactive substances, and creating hydrogel patch with certain ingredients. The quality of the patch is evaluated by different matrices, and the integrity of the bioactive components is guaranteed by characterisation using Fourier- transform infrared spectroscopy. The potential of Allium Ascalonicum to fight infections and the creation of unique hydrogel patch for skin and tissue infections are highlighted in the study's conclusion, adding to the body of knowledge on natural solution to problems with conventional medicine.

Key Words: Allium Ascalonicum, Extract, Skin infections, Antimicrobial activity

I. INTRODUCTION

The body's integrity depends on healing of skin infection, since the skin serves as the principle defence against outside influences. This barrier is compromised by skin damage from cuts, burns and incisions, requiring efficient therapies. Immune cells such as neutrophils, macrophages, and leukocytes control the healing process, which includes homeostasis, inflammation, proliferation and remodelling.

The limits of current treatments include immunogenic responses, graft rejection and expensive costs, current treatment includes medicines, ointments and dressings such as collagen membranes and gauze. Large, misshapen infections treated with conventional methods can leave tissue loss and scaring. Natural healing is provided by innovations like amniotic membrane and acellular tissue allografts although they are constrained by the lack of donor tissue

Hydrogels offer potentially effective remedy by creating a damp atmosphere that facilitates quick, infection free-healing. These polymers can be designed for regulated medication distribution, which lessens the need for frequent dressing changes and improves therapeutic efficacy. They can also store sizable volumes of water and biological fluids.[1]

Shallots (Allium Ascalonicum) possess antibacterial and antioxidant properties since they contain bioactive components such as thiosulfate and allicin. Shallots possess these qualities, which make them effective against microorganisms that are often associated with skin and tissue infections, such as Candida albicans, Pseudomonas aeruginosa, and Staphylococcus aureus.[2]

An inventive method of skin care is provided by combining hydrogel dressings with shallots, antibacterial characteristics. By preserving a moist, infection-free atmosphere and utilizing shallot's inherent healing qualities, this two-prolonged approach can improve healing. To enhance treatment of skin infection and patient outcomes, this publication investigates the possibility of synergy between hydrogel dressings and shallots.[2]

II. MATERIALS AND METHODS

Collection and Authentication

Shallot plant were collected from the garden area of Kalpetta, Wayanad and authentified by

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A.K Pradeep, assistant professor, Department of botany, University of Calicut, for the purpose of project work.

Extraction

500g of shallot bulbs were carefully cleaned and forcefully mashed in the kitchen mixer. After mashing the shallot, and add 500 ml of purified water, and let it soak for 5 hours while being stirred with a magnetic stirrer. The suspension was then passed through Whatman filter paper for filtering. After combining the resulting water extract with ethyl acetate in 50:50 ratio, it was agitated for 10 minutes using a magnetic stirrer. Using a separating funnel, the upper organic layer was separated, and the mixture was centrifuged at 5000 rpm. After that, the ethyl acetate layer was taken off and put in a flask. Repeat this process for 3 times, pool the extract and dry in a rota evaporator at 500℃.[3]

EVALUATION OF ALLIUM ASCALONICUM EXTRACT

Chemical Test for Shallot Extract

To determine the chemical components, a preliminary phytochemical screening was carried out. Two sulphur tests are conducted because the components of the shallots are primarily sulphurous.

Sulphur test/ Lead acetate test: In a test tube, 2ml if extract was added, along with 3 to 4drops of lead acetate and 2 drops of 40% of sodium hydroxide. And the mixture was continued to stir until the precipitate is dissolved. After 2 minutes of boiling, the test tube was cooled. Precipitate with brownish black colour indicates the presence of sulphur compound.

Sodium Nitroprusside test: A test tube containing 2 ml of extract was filled with 3 to 4 drops sodium nitroprusside. 3 or 4 drops of 40% of sodium hydroxide were added to the medium to turn it alkaline. Appearance of violet colour indicates the presence of sulphur compound.[4]

Determination of Antibacterial Activity

Nutrient agar plates were made by putting the medium onto sterile Petri dishes as per the manufacturer's instructions and allowing it to settle, resulting in a homogeneous layer that was around 4-5 mm thick. Next, broth was inoculated with a single colony of Staphylococcus aureus bacteria to create a bacterial suspension. To establish a consistent bacterial lawn, the bacterial suspension was evenly distributed over the whole surface of the agar plate using a sterile cotton swab.

Following the preparation of the agar plates, wells measuring between 6 and 8 mm in diameter were made in the agar using a sterile cork borer or well punch. The wells were not too near to the edge or to one another, and they were evenly spaced. The test material was then added to each well in a precise volume of 100 µL using a sterile pipette. Every plate had two controls: sterile water for the negative control and cefotaxime sodium for the positive control.

Following the addition of the test materials, the plates were allowed to sit for one to two hours at room temperature so that the materials could permeate into the agar. Next, for 18 to 24 hours, the plates were incubated at the proper temperature, which is typically 37°C for most bacteria. Following incubation, the plates were inspected to look for clear areas surrounding the wells where the growth of bacteria had been suppressed. A ruler or callipers were used to measure the diameter of these zones of inhibition, and the results were recorded.

The test substance's antibacterial activity was demonstrated by the existence of a clear zone surrounding a well. Generally speaking, larger zones indicated higher antibacterial activity. The relative effectiveness of the test drugs was determined by comparing these zones with those surrounding the positive and negative controls. To prevent contamination, it was essential to carry out all procedures in a sterile manner. Additionally, repeating the experiment in three copies would guarantee precision and reproducibility.[5,6]

Determination of MIC

Extract dilution in series: Using aseptic method, transfer 9 mL of medium into five test tubes using sterile 10 mL pipettes or sterile automatic dispensers. Indicate the dilution factor on tubes 10^{-1} to 10^{-6} labels. Gently mix 1 milliliter of extract (10^{-3}) into the first tube aseptically. Transfer 1 milliliter of this dilution to the subsequent tube (10^{-2}), and gently stir. Do the same with the remaining tubes (10^{-1} to 10^{-6}). The minimum inhibitory concentration was performed using well diffusion method.[7]

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PREPARATION OF HYDROGEL PATCH

Formulation:

Sl. No.	Ingredients	F1	F2	F3
1.	Sodium alginate (g)	0.1	0.15	0.1
2.	Hydroxyethyl cellulose (g)	0.03	0.02	0.03
3.	N, N'-Methylenebisacrylamide (g)	0.1	0.2	0.2
4.	Ethanol (ml)	4	4	4
5.	Ammonium persulfate (g)	0.1	0.1	0.1
6.	Acrylic acid (ml)	4	4	4
7.	Distilled water (ml)	15	15	15

Method of Preparation:

To produce the intended outcome, the process goes through multiple stages. Phase 1 involves dissolving the recommended amount of sodium alginate in 5 ml of distilled water and stirring the mixture for 20 minutes at 37℃ to create a uniform mixture. Phase 2 involves making a translucent solution, dissolve hydroxyethyl cellulose separately in 5 ml of purified water and stir at 90℃. In phase 3, dissolve the specified amount of methylene bisacrylamide and ammonium persulfate in 5 ml of distilled water in a separate beaker using a hot plate magnetic stirrer set to 37℃ to form a transparent solution. Pour the specified amount of acrylic acid to a different beaker during Phase 4. Drop by drop, add the prepared solutions to the cooled polymer mixture in Phase 5. Using an electric water bath set at 50℃ for 2 hours and 65 ℃ for the entire night, phase six is carefully putting the reaction mixture into labelled Petri dishes, covering them with aluminium foil, and starting the reaction. In phase 7, the patches should be taken out of the Petri plates after a day and carefully cleaned with a water-ethanol solution to get rid of any contaminants and uncross-linked chemicals. The prepared hydrogel patches are impregnated with allium ascalonicum extract.[8]

EVALUATION OF HYDROGEL PATCH

1. **Thickness measurement:**

Thickness of patch was measured at different point using vernier callipers. The average of 3 readings were taken.[9]

2. **Weight Variation:**

The patches were selected from each formula and weighed one by one and average weight and percentage weight variation were calculated. [9,10]

Average weight = Total weight of patchesTotal number of patches (1)

% Weight variation = Weight of individual patch – Average weightAverage Weight (2)

3. **Sol-Gel Fraction:**

Fraction of Sol-Gel The hydrogel patches made of polymeric cross-linking were dried at 40 °C in an electric oven. Following drying, each patch was separately placed into labeled beakers with 200 mL of distilled water, and then weighed using an electronic balance. Uncross-linked polymer components had to be periodically stirred out of the patch structure. Hydrogel patches were taken out of the distilled water after 24 hours, transferred into Petri dishes with labels, and dried in an oven to achieve a consistent weight. Equations (3) and (4) were used to calculate the gel and sol fractions, respectively.[12,13]

Gel (%) = (Wg/W0) × 100 (3)

Sol (%) = 100 – Gel (%) (4)

W0 = Initial weight of the dried patch

Wg = weight of the extracted dried patch

4. **Stability testing:**

Stability testing was done at various temperature of $10^{O}C$, $20^{O}C$, $30^{O}C$, $40^{O}C$, $50^{O}C$,$60^{O}C$. The visual testing was done at each temperature. The formulation was found to be stable and good till $50^{O}C$. The formulation was found to be unstable above the $60^{O}C$.[14]

5. **Antibacterial activity of Hydrogel Patch:**

The antibacterial activity of the hydrogel patch was performed using disc diffusion method. Following the addition of the prepared hydrogel patches, the plates were allowed to sit for one to two hours at room temperature so that the materials could permeate into the agar. Next, for 18 to 24 hours, the plates were incubated at the proper

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temperature, which is typically 37°C for most bacteria. Following incubation, the plates were inspected to look for clear areas surrounding the wells where the growth of bacteria had been suppressed. A ruler or callipers was used to measure the diameter of these zones of inhibition, and the results were recorded. The antibacterial activity of the hydrogel patches were demonstrated by the existence of a clear zone surrounding a well. Larger zones indicated higher antibacterial activity. The relative effectiveness of the test patches were determined by comparing these zones with those surrounding the positive and negative controls.[5,6]

III. RESULT AND DISCUSSIONS

EVALUATION TEST FOR EXTRACT

1. Chemical test for shallot extract:

The sulphur/Lead acetate test for shallot extract confirms the presence of sulphur in the prepared extract of shallot. Sodium nitroprusside test for shallot extract confirms the presence of sulphur in the prepared extract of shallot. The chemical tests confirmed the presence of sulphur containing compound namely allicin.

2. Determination of Antibacterial Activity:

Zone of inhibition was observed for the prepared Allium ascolanicum extracts and hence it was proved to have antibacterial activity.

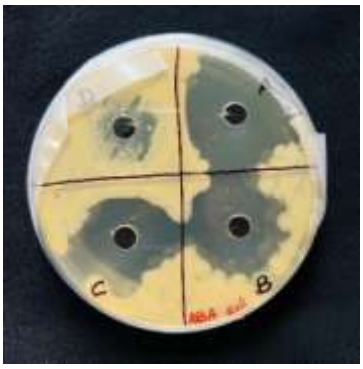

Fig. 1

3. Determination of MIC: The minimum inhibitory concentration of the prepared extracts were determined and the concentration of 10^{-3} (B) was found to have antibacterial activity in the minimum concentration and hence the MIC of the extract was found to be 0.01µl/ml.

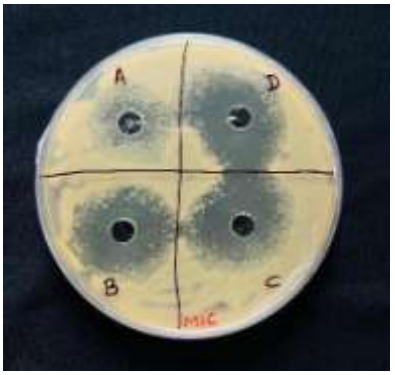

Fig. 2

EVALUATION TEST FOR HYDROGEL PATCHES:

1. Thickness measurement:

Thickness of patch was measured at different point using vernier caliber. The optimum thickness of 0.11 mm was found for F3 formulation.

2. Weight variation:

The selected patches from each formula are weighed one by one and its percentage weight variation were found to be 3.9, 1.9, 0 for F1, F2, F3 respectively. The formulation F3 was found to have least weight variation and hence opted as best formulation.

3. Folding endurance:

The folding endurance of the formulations F1 to F3 was determined and was found to be varying from 45 to 50. Accordingly, the formulation F3 exhibited enhanced folding endurance compared to all other formulations.

4. Sol-gel fraction:

Sol-Gel fractions were calculated using respective equations. The Sol% were found to be 20, 16.6 15.2 and Gel% were found to be 80, 83.4, 84.8 for F1, F2 and F3 respectively. Hence, from the observations it was optimized that F3 was having more sol-gel fraction compared to other formulations.

5. Stability testing:

Stability testing was done at various temperature of 10^{O}C, 20^{O}C, 30^{O}C, 40^{O}C, 50^{O}C,60^{O}C. The visual testing was done at each temperature. The formulation was found to be stable and good till 50^{O}C. All the formulations were found to be unstable above the 60^{O}C.

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6. Antibacterial Activity of Hydrogel Patches:

The antibacterial activity of hydrogel patches were determined by disc diffusion method and all the patches showed profound antibacterial activity and hence we can conclude that the hydrogel patches impregnated with Allium ascolanicum extract is having antibacterial activity.

Fig. 3

IV. CONCLUSION:

The current study frames a conclusion that Allium ascolanicum extract is having profound antibacterial activity. The chemical studies verified the presence of sulphur compound which can be allicin which have been already proved having antimicrobial activity. The antibacterial activity of the extract was determined using Staphylococcus aureus bacteria which was found to be the most common bacteria causing skin and wound infections. The extract were impregnated in hydrogel patches which can be emphasised for prolonged antibacterial activity and hence can be utilized for the treatment of skin and wound infections. Thus, the antimicrobial hydrogel patches prepared with Allium ascalonicum extract was found to be a better treatment option for skin and wound infections. The study can be further advanced by isolating Allicin from the Allium ascalonicum extract and thereby preparing hydrogel patches with the same.

REFERENCE:

[1]. Morris CA, Avorn J. Internet marketing of herbal products. J Am Med Assoc. (2003) 290:1505– 9. doi: 10.1001/jama.290.11.1505.

[2]. Ali, S.; Shabbir, M.; Shahid, N. The structure of skin and transdermal drug delivery system— A review. Res. J. Pharm. Technol.2015, 8, 103–109.

[3]. Amin M, Segatoleslami S, Hashemzadeh M. Antimycobacterial activity of partial purified extract of Allium ascalonicum. Jundishapur J Microbiol. 2009;2(4):144-147.

[4]. Singh S, Jha A, Acharya S, et al.Determination and estimation of allicin inallium sativum. J Evolution Med Dent Sci 2020;9(49):3711-3715, DOI: 10.14260/jemds/2020/815.

[5]. Ivanova A, Mikhova B, Najdenski H, Tsvetkova I, Kostova I: Chemical composition and antimicrobial activity of wild garlic Allium ursinum of Bulgarian origin. Nat Prod Comm 2009, 4:1059-1062.

[6]. Fujisawa H, Watanabe K, Suma K, Origuchi K, Matsufuji H, Seki T, Ariga T: Antibacterial potential of garlic-derived allicin and its cancellation by sulfhydryl compounds. Biosci BiotechnolBiochem 2009, 73:1948-1955.

[7]. Barbu IA, Carpa R. Phytochemical characterization and antimicrobial activity of several Allium extracts. Molecules. 2023;28(10):3980.

[8]. Ebnesajjad, S. 8—Characteristics of Adhesive Materials. In Handbook of Adhesives and Surface Preparation; Ebnesajjad, S., Ed.; William Andrew Publishing: Oxford, UK, 2011; pp. 137–183.

[9]. El-Gendy N, Abdelbary G, El-Komy M, Saafan A. Design and evaluation of a bioadhesive patch for topical delivery of gentamicin sulphate. Curr Drug Delivery. 2009;6(1):50–7

[10]. Darwhekar G, Jain DK, Patidar VK. Formulation and evaluation of transdermal drug delivery system of clopidogrel bisulfate. Asian J Pharm Life Sci ISSN. 2011;2231:4423.

[11]. Takeuchi Y, Ikeda N, Tahara K, Takeuchi H. Mechanical characteristics of orally disintegrating films: Comparison of

folding endurance and tensile properties. Int J Pharm. 2020; 589:119876.

[12]. Ollinson MM. Sol-gel strategies for the preparation of selective materials for chemical analysis. Crit Rev Anal Chem. 1999;29(4):289–311.

[13]. Hwang, M.-R.; Kim, J.O.; Lee, J.H.; Kim, Y.I.; Kim, J.H.; Chang, S.W.; Jin, S.G.; Kim, J.A.; Lyoo, W.S.; Han, S.S. Gentamicinloaded wound dressing with polyvinyl alcohol/dextran hydrogel: Gel characterization and in vivo healing evaluation. AAPS PharmSciTech 2010, 11, 1092– 1103.

[14]. Reddy SH, Manimaran V. Formulation and development of doxycycline hyclate loaded chitosan nanoparticles crosslinked topical hydrogel patches for healing diabetic foot ulcer. J Med Chem Sci. 2024;7(6):753-776.