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Formulation And Evaluation of plant-based Hydrogel for Antimicrobial Activity

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ABSTRACT

The study was aimed to develop and evaluate a plant-based hydrogel incorporating chitosan polymer for its *in-vitro* antibacterial activity. The hydrogel was prepared by using chitosan polymer, methyl paraben, propyl paraben, glycerin, acetic acid, triethanolamine, moringa oleifera leaf extract and distilled water. Triethanolamine was gradually added drop by drop to maintain the pH of skin. The hydrogel formulations were assessed based on various parameters, including physical appearance, homogeneity, viscosity, extrudability, pH, spreadability, moisture content and antimicrobial efficacy. The formulated gel was dark brown, homogenous and pH ranges from 6.24 to 6.29 which is close to the skin pH. Formulation F4 showed acceptable rheological property with applicable spreadability, extrudability, moisture content and *in- vitro* antibacterial activity. The phytochemical screening of methanolic extract revealed the presence of various secondary metabolites such as flavonoids, alkaloids, glycosides, terpenoids, saponins, phenols and tannins. Antimicrobial activity was tested using the agar well diffusion method. The m. oleifera plant was recognized as a rich source of natural antimicrobial agents due to its phytochemical properties. The antimicrobial activity of the test sample was quantified by measuring the diameter of the inhibition zone in millimeters. The formulation F4 shows highest inhibition zone of 17mm at the highest concentration (100mg/ml). Antimicrobial screening revealed that the crude methanolic extract exhibited antimicrobial activity against Staphylococcus epidermidis.

Keywords: *Hydrogel, Chitosan, Phytochemical screening, Anti-microbial activity.*

1. INTRODUCTION

1.1 Herbal Medicine

The significance of herbal medicine practices is represented by the fact that nearly 80% of the people in developing nations believes on conventional medicine for their primary healthcare needs. Therefore, it is medically and economically crucial, to scientifically assess the safety and effectiveness of herbal products and medicinal preparations (Mosihuzzaman et al. 2008).

To improve health, herbal medicine products are often used as dietary supplements and are available in forms like capsules, tablets, teas, extracts, powders, and fresh or dried plants. While traditionally regarded as safe, they are increasingly consumed without prescription (Modak, Manisha et al. 2007). Traditional medicine includes herbal drugs made from aerial parts, fruits, flowers, leaves, stems, seeds, and underground parts like tubers, and rhizomes, roots, bulbs (Muyumba et al. 2021).

Herbal medicine has been used for centuries and is widely accepted due to its better tolerance among patients. Long-term and regular usage of herbal medicines may demonstrate their safety and efficacy (Wani et al. 2007).

1.2 Chitosan

Natural polymer hydrogels have acquired more attention compared to synthetic polymer hydrogels due to their advantages such as low toxicity, excellent biocompatibility, easy biodegradability, abundant availability, and affordability. A naturally occurring polysaccharide, chitosan consists of glucosamine and N-acetyl glucosamine units joined through β-(1-4) glycosidic linkages (Fu J et al. 2018).

Chitosan originates from chitin, which is found in the exoskeletons of crustaceans like shrimp and crabs and is the second most abundant natural polymer after cellulose (Singla and Chawla 2001). Chitosan can be formulated into various forms, including hydrogels, xerogels, beads, powders, tablets, capsules, films, microparticles, microspheres, nanofibrils, textile fibres, and inorganic composites (Öztürk B. 2011).

Additionally, chitosan is metabolised by specific human enzymes, particularly lysozyme, and is regarded as biodegradable. In conclusion, Due to its abundance, low production cost, and eco-friendly nature, chitosan is a popular choice for use in hydrogels for medical and pharmaceutical use (Berger et al. 2004).

Chitosan is commonly used to synthesize hydrogels due to its strong crosslinking ability, along with its biodegradability, biocompatibility, and non-toxicity, making chitosan-based hydrogels highly attractive for use in biomedical and tissue engineering fields (De Azevedo et al. 2015).

The mechanism behind chitosan's antibacterial activity involves the positively charged chitosan moiety (NH_3^+ group) can interact with the negatively charged bacterial cell surface, disrupting the cell membrane and ultimately leading to cell death (Venkatesan et al. 2014).

1.3 Hydrogel

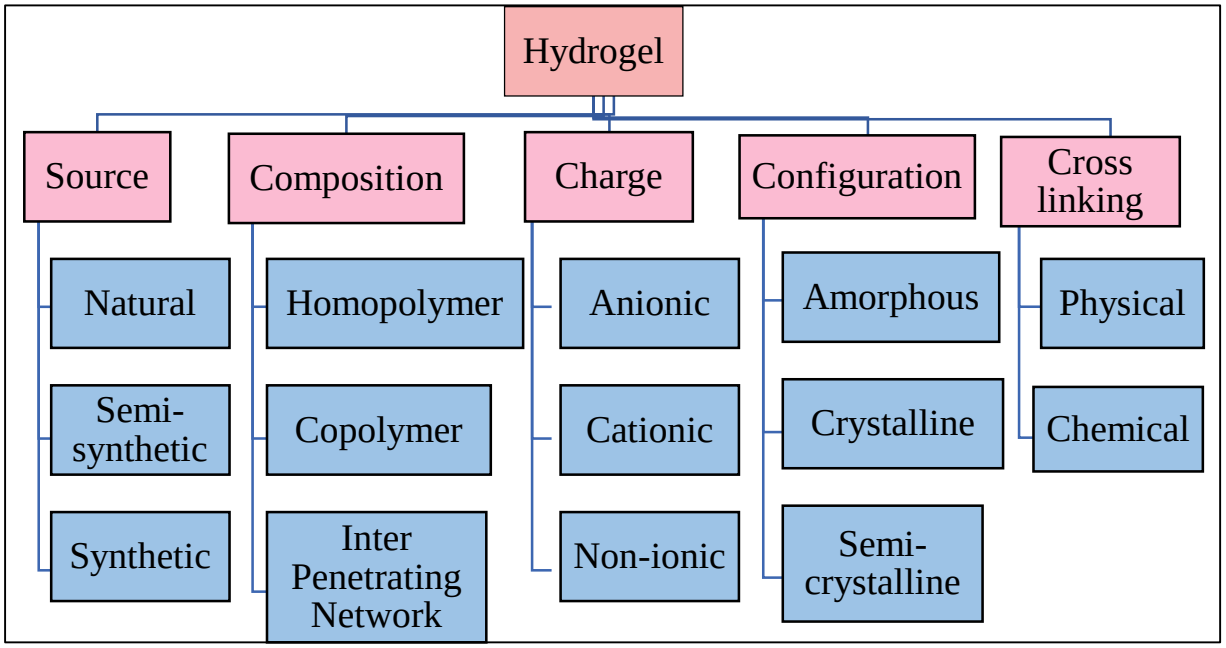
Hydrogels are 3D polymeric networks consisting of natural or synthetic materials, characterized by their high-water content, which gives them remarkable flexibility. In physiological conditions, Hydrogels are capable of retaining and absorbing a large volume of water or biological fluids and their soft, tissue like texture makes them perfect for a wide variety of applications (Ullah et al. 2015).

Due to the presence of hydrophilic functional groups, hydrogels can absorb water, allowing them to swell by tens or hundreds of times their mass. The synthesis of chitosan-based hydrogels can be achieved using several techniques, including physical interaction and chemical cross-linking. Chemical cross-linking produces a highly stable and permanent structure, whereas physical cross-linking leads to a dynamic structure that is influenced by environmental conditions (Thirupathi et al. 2022).

The unique properties, such as excellent biocompatibility, versatility, efficient responsiveness to stimuli, high flexibility, softness, degradability, and high-water content make hydrogel well-suited for a wide range of applications, ranging from industrial to biological fields (Khan et al. 2024).

Hydrogels have a wide range of clinical applications, including angiogenesis, wound healing, liver regeneration, drug delivery etc. Hydrogels also occur naturally in the human body, where they are found in mucus, blood clots, vitreous humour, cartilage, and tendons (Khandan et al. 2017).

1.4 Classification of Hydrogel



1.5 Application of Hydrogel

Wound Healing

Drug Delivery in GI Tract

Contact lenses

Tissue Engineering

Hydrogel for Gene delivery

Transdermal Drug Delivery

Protein drug delivery

1.6 Plant Profile

Moringa oleifera

Biological source

Moringa oleifera is a small, fast-growing, drought-resistance tree of the family Moringaceae that usually grows upto 10-12m in its height. Moringa oleifera is a tropical tree native to India, Pakistan Bangladesh and Afghanistan.

Chemical Constituent

Moringa oleifera, rich in protein, vitamins, minerals, and carbohydrates, has long been used as a natural medicinal plant that can potentially reduce malnutrition. With their abundant protein and high digestibility, moringa leaves serve as an excellent source of nutrition. Vitamins are essential as they play a crucial role in the energy metabolism of the animal body. Vitamin deficiencies can result in common diseases such as beriberi, rickets, and scurvy. Moringa oleifera is a rich source of vitamins A (beta-carotene), B (folic acid, pyridoxine, and nicotinic acid), C, D, and E. A diverse range of phytochemicals, such as flavonoids, alkaloids, saponins, sterols, tannins, and terpenoids are present in Moringa leaves **(Islam Z. et al. 2021).**

Medicinal Uses

Antioxidant activity

Antimicrobial activity

Anti-inflammatory activity

Ant cancerous activity

Anti-hyperglycaemic (antidiabetic)

Hypolipidemic

Hepato-protective

2. MATERIALS AND METHODS

2.1 Collection and Authentication of the plant materials

Based on a thorough study of ethnopharmacological data from the botanical garden, fresh leaves of completely grown plant (M. oleifera) were collected from botanical garden of CSIR-National Botanical Research Institute, Lucknow and further identified and authenticated by Dr. Sharad Srivastava, Chief Scientist and Head of Pharmacognosy Division, CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh.

After its collection, after being cleaned with water that had been distilled, the plant leaves were dried in the shade till constant weight of leaves was achieved. The crushed leaves was subjected for extraction.

2.2 Preparation of methanolic extract

In cleaned and dried conical flask, 5 gm dried plant material (M. oleifera) were filled and 100 ml of methanol was poured into the flask and shake very gently then left for overnight. Next day the extract was filtered by using filter paper. Now pour the filtrates into a round bottom flask and evaporated by using rotavapor. The procedure was repeated 2 to 3 times with 100 ml methanol. The extract obtained by above procedure is known as methanolic extract.

2.3 Materials

All the chemicals and other instruments were provided by Pharmacognosy division, CSIR- National Botanical Research Institute, Lucknow.

2.4 Phytochemical Screening of Extract

Phytochemicals can be extracted from plant materials using various techniques. Key phytochemicals include alkaloids, flavonoids, glycosides, tannins, saponins, phenolics, and terpenes, which are distributed across different plant parts.

2.5 Formulation of chitosan hydrogel

A beaker was filled with 40 ml of distilled water, and 0.4 gm of acetic acid was added, then the beaker was set aside. In another beaker, 10 ml of water was taken and heated on a water bath to dissolve methyl and propyl paraben. To this beaker, 0.05 gm of methyl paraben and 0.025 gm of propyl paraben were added. Once the methyl and propyl parabens were

completely dissolved and the solution had cooled, 1 gm of glycerine was added. The solution from the second beaker was then poured into the first beaker, and the mixture was stirred continuously for 10 minutes with a mechanical stirrer at 1000 rpm. During this stirring process, 1 gm of chitosan was gradually added. After the addition of chitosan, a pre-measured amount of extract was added to this solution and carefully stirred. Triethanolamine (TEA) was added drop wise while swirling continuously to create hydrogel with desired viscosity and consistency. After mixing, the hydrogel was set aside for 24 hours to release any entrapped air. As a result of this procedure, a thick, whitish, cloudy hydrogel formulation was obtained.

Table 1: Formulation Table

Ingredients	**F1**	**F2**	**F3**	**F4**
M. oleifera extract	25	50	75	100
Chitosan	1.0	1.0	1.0	1.0
Acetic acid	0.4	0.4	0.4	0.4
Glycerine	2.0	2.0	2.0	2.0
Methyl paraben	0.05	0.05	0.05	0.05
Propyl paraben	0.025	0.025	0.025	0.025
Triethanolamine	q.s.	q.s.	q.s.	q.s.
Water	q.s.	q.s.	q.s.	q.s.

2.6 *In vitro* anti-microbial activity

The *in vitro* anti-microbial activity was evaluated by measuring the zone of inhibition on an inoculated agar plate. The zone of inhibition refers to area surrounding the well containing the antimicrobial agent on the agar surface. It indicates the agent's ability to suppress microorganism growth. The greater the diameter of this zone, the more potent the antimicrobial activity.

2.7 Preparation of media and its sterilisation

The required amount of ingredients was taken in flasks and dissolved in distilled water. All equipment and media involved in the experiment were sterilized at 121˚C, at 15 psi pressure for 1 hour. The sterilized media were transferred to petri plates under a biological safety cabinet and allowed to solidify. The plates were then turned upside down in an incubator at 37˚C for 24 hours.

2.8 Anti-bacterial activity

Anti-bacterial activity of plant extract was tested against bacterial pathogen, Staphylococcus epidermidis using the agar well diffusion method. To prepare the dilution, extracts were dissolved in DMSO. The first step involved reactivating bacterial stock cultures by inoculating them in nutrient-agar media and incubating at 37ºC for 18 hours. Nutrient agar plates were prepared, and wells were then created in the nutrient-agar media. Each plate was inoculated with 100 µl of 18-hour-old bacterial cultures (10^4 cfu) and spread evenly across the surface by using a sterile spreader. With the help of sterilised cork borer, uniform wells were made into the dried nutrient-agar media plates. After 20 minutes, every well was filled with methanolic plant extract. The control well with DMSO was also prepared. The plates were placed in an incubator for 24 hours at 37˚C and the inhibition zone diameter was recorded in mm.

2.9 Evaluation of Hydrogel

Evaluation parameters such as colour, consistency, homogeneity and physical appearance of the formulation were inspected through visual inspection.

1. pH determination

The pH of each concentration (25mg/ml,50mg/ml,75mg/ml,100mg/ml) of hydrogel was measured with the help of digital pH meter. 2.5 gm of hydrogel was dissolved in 25 ml of purified water. Prior to each use, the pH meter was calibrated with

standard buffer at pH 4.0, 7.0, and 9.0. The electrode was inserted into the hydrogel sample to take the reading. Triplicate measurements were taken to determine the pH of the formulation, and the mean values were calculated.

2. Viscosity

The viscosity of the hydrogel was evaluated with the help of Brookfield viscometer. The spindle no.7 was dipped in hydrogel and rotated at a speed of 0.3, 0.6, 1.5, 3, 6, 12, 30, 60 rpm at room temperature. The hydrogel was filled in a wide mouth container, ensuring the spindle of the viscometer could be fully immersed. At every speed, the dials reading was noted. The viscosity of hydrogel was obtained by multiplication of the dial reading with the factor given in the Brookfield Viscometer catalogue.

3. Spreadability

To determine the spreadability of the gel formulations, 1 g of gel was placed between two horizontal plates (20 cm × 20 cm). To test the spreadability, the upper slide was loaded with a 100 g weight for 60 seconds ensuring the gel forming thin, uniform layer and the spreading diameter of the hydrogel was then measured. The result was obtained using the following formula;

$$S = M \times L/T$$

where, S = spreadability, M = weight tied to upper slide, L = length of the glass slide (7.5 cm), T = time taken in

4. Extrudability

Collapsible aluminum tubes with caps were used to fill the hydrogel formulations, and the tubes were sealed by crimping the ends. Two glass slides were used to sandwich the tubes, which were then clamped together with a 500 g weight placed on top. The extruded hydrogel was collected and weighed after the cap was removed. The amount of extruded hydrogel, expressed as a percentage was calculated, where

>90% is considered excellent,

>80% is good, and

>70% is fair.

5. Moisture content

Measure the weight of an empty Petri dish using an analytical balance. Add a known volume of hydrogel to the dish and weigh it again then place the dish in a drying oven at 105°C for three hours. Keep drying the sample until the weight remains constant. Then allow the sample to cool in a desiccator and weigh the dish again to obtain the final mass. The percentage of moisture content were then determined by taking the difference between the initial and final weights and dividing by the final weight.

Moisture content (%) = $(W_i - W_d) / W_d \times 100$

Where, W_i is the initial weight of hydrogel and W_d is the final weight of hydrogel.

3. RESULT AND DISCUSSION

Four formulations of chitosan-based hydrogel were formulated using different concentration of extract. All formulations were evaluated for their appearance, homogeneity, color, odor, pH, spreadability, extrudability, viscosity, moisture content and *in-vitro* antimicrobial activity.

3.1 Extractive yield of Plant material

The percentage yield is expressed as the ratio of actual yield to theoretical yield in percentage form. It is determined by multiplying the experimental yield by 100% and dividing it by the theoretical value. The percentage yield of methanolic extract of plant (M.oleifera) was found to be 24.14%.

3.2 Phytochemical analysis

Phytochemical analysis of plant (M. oleifera) revealed the presence of alkaloids, flavonoids, glycosides, terpenoids, saponin, phenols and tannin. The results obtained were summarised in table 2.

Table 2: Phytochemical Screening of extract

S. No.	Test	Plant extract
1	Alkaloids	+

2	Flavonoids	+
3	Glycosides	+
4	Terpenoids	+
5	Saponins	+
6	Phenols and Tannins	+

3.3 *In-vitro* anti-microbial activity of plant extract

The antimicrobial activity of M. oleifera leaf extract (methanolic extract) was studied at concentration (1000µg/ml) against Staphylococcus epidermidis bacteria by Agar well diffusion method. *In-vitro* anti-microbial activity revealed clear zone of inhibition of plant extract and their activity against Staphylococcus epidermidis bacteria. Result of anti-microbial activity and zone of inhibition was shown in below table.

Table 3: Zone of inhibition of plant extract

S. No.	Concentration	Zone of inhibition (Mean ± SD)
1	**10mg/ml**	9 ± 1.15
2	**50mg/ml**	19 ± 0.57
3	**100mg/ml**	23 ± 1

3.4 *In-vitro* anti-microbial activity of all formulation at different concentration

On increasing the concentration of plant extract, antimicrobial activity of hydrogel increases. Among the four formulations, formulation F4 shows highest zone of inhibition (17mm) compared to other formulation, thus showing best antimicrobial activity against staphylococcus epidermidis bacteria.

Table 4: Zone of inhibition of formulation

Formulation	Concentration	Zone of inhibition (Mean ± SD)
F1	**25mg/ml**	Nil
F2	**50mg/ml**	Nil
F3	**75mg/ml**	16 ± 5.6
F4	**100mg/ml**	17 ± 6.6

3.5 Evaluation of Hydrogel

1. Physical evaluation

The physical parameters such as appearance, homogeneity, colour and consistency of the formulation were checked by visual examination and results were given in the table 5.

Table 5: Physical evaluation of all formulation

Formulation	Color	Feel on application	Consistency	Homogeneity	Physical appearance
F1	Dark brown	Cooling effect	Good	Homogenous	Opaque
F2	Dark brown	Cooling effect	Good	Homogenous	Opaque
F3	Dark brown	Cooling effect	Good	Slightly Homogenous	Opaque
F4	Dark brown	Cooling effect	Good	Homogenous	Opaque

2. pH Determination

The determination of pH is an important criterion for topical medical application because if it deviates from normal skin pH, then it may cause skin irritation. The pH of the formulation 4 was found to be 6.29, which is close to the skin pH. Therefore, the topical hydrogel formulation may not irritate the skin when administered topically. The measurement of each formulation was done and the result were shown on the table 6.

Table 6: pH of all formulation

S. No.	Formulation	pH (Mean ± SD)
1	F1	6.24 ± 0.04
2	F2	6.25 ± 0.17
3	F3	6.25 ± 0.04
4	F4	6.29 ± 0.07

3. Viscosity

Viscosity of different formulation was estimated through Brookfield viscometer with spindle no. 7 at 100 rpm. The viscosity of the samples was measured under room temperature. (25 – 27˚C). The viscosity of herbal hydrogel is a key factor in determining its mechanical and physical properties such as spreadibility. Viscosity of all four formulation was different and F4 formulation have best viscosity than other formulation and it is reported in table 7.

Table 7: Viscosity of all Formulation

S. No.	Formulation	Viscosity
1.	F1	3048
2.	F2	3201
3.	F3	3126
4.	F4	3287

4. Spreadability

Spreadibility of hydrogel is necessary to the application process; if spreadibility is poor, it eventually shorten the time that the drug remains on the skin, which can result in poor absorption and bioavailability. The spreadability of F4 formulation was found to be 35.25 ± 4.40 g/cm/sec, which is good among all formulations. Spredability of gel formulation is reported in table 8.

Table 8: Spreadability of all formulation

S. No.	Formulation	Spreadability (Mean ± SD)
1	F1	55.41 ± 5.31
2	F2	50.97 ± 2.03
3	F3	44.26 ± 4.51
4	F4	35.25 ± 4.40

5. Extrudability

The ability to extrude the hydrogel from the tube is significant for its use and patient acceptance. Hydrogels that are too thick may not extrude properly from the tube, while hydrogels with low viscosity may flow too rapidly, so the hydrogel needs to have the correct consistency for extrusion. Extrudability of all hydrogel formulation was found to be good. Table 9 shows that Formulation F4 have better Extrudability compared to all formulation.

Table 9: Extrudability of all formulation

S. No.	Formulation	Percentage Extrudability (%)
1	F1	82.34
2	F2	86.62
3	F3	85.60
4	F4	90.40

6. Moisture Content

To determine the moisture content, the hydrogel was kept in a desiccator with activated silica for 24 hours. The weight difference in relation to the final weight was used to determine the percentage of moisture content. The moisture content of chitosan-based hydrogels typically ranges from 70% to over 90%. The moisture content results for the various formulations are presented in Table 13. The F4 formulation showed the best moisture content when compared to the other formulations.

Table 10: Moisture Content of all formulation

S. No.	Formulation	Moisture Content
1.	F1	96.32 %
2.	F2	96.96 %
3.	F3	96.25 %
4.	F4	97.41 %

4. CONCLUSION

From above result it was conclude that hydrogel formulation prepared with chitosan using M. oleifera leaf extract showed acceptable physical properties, good stability and is quite effective as an antibacterial. Hydrogels are getting more popular nowadays because they are more stable and also provide controlled release than other semi-solid preparations like cream, ointments, pastes etc. The hydrogel was prepared by using different concentration of plant extract and physical evaluation of all formulation were done.The hydrogel formulation enhances absorption characteristics and hence improving the bioavailability of the drug. Antibacterial hydrogels find applications in wound dressings, urinary tract coatings, infections related to catheters, and gastrointestinal infections, among others. Phytochemical screening confirmed the presence of alkaloids, flavanoids, carbohydrates, glycosides, phenols and tannins. The anti microbial activity analysis indicates that plant extract is highly effective against S. epidermidis bacteria. Formulation F4 showed highest zone of inhibition at 1000µg/ml.

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