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Formulation and evaluation of hydrogel incorporating bay leaf extract for treatment of dermatitis

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Abstract
Dermatitis, a common inflammatory skin condition, poses significant challenges in treatment due to its complex etiology and varied clinical manifestations. In recent years, natural extracts have garnered attention for their potential therapeutic properties in dermatological applications. This study focuses on the development of a hydrogel formulation containing bay leaves extract for the treatment of dermatitis. The hydrogel was formulated using biocompatible polymers and incorporated with bay leaves extract known for its anti-inflammatory, antioxidant, and antimicrobial properties. Physicochemical characterization revealed a stable hydrogel with desirable rheological properties, ensuring ease of application and adherence to the skin. Overall, the developed hydrogel formulation holds promise as a novel therapeutic approach for dermatitis, offering potential benefits in alleviating symptoms and promoting skin healing. Further clinical studies are warranted to validate its efficacy and safety in human subjects, thereby advancing its translation into clinical practice for the management of dermatological disorders.

Keywords: Hydrogel, dermatitis, etiology, polymers

Introduction
Hydrogels are three dimensional networks composed of hydrophobic polymers synthesized by crosslinking water-soluble polymers. Hydrogels can retain a large quantity of water within their network without disturbing their original structure. This imparts flexibility and swelling properties to the hydrogel structure. Dermatitis is a challenging skin condition characterized by inflammation and discomfort. Conventional treatments often come with drawbacks, prompting exploration into alternative therapies. Bay leaves, known for their anti-inflammatory properties, hold promise as a natural remedy. Hydrogels, due to their moisture-retaining capabilities, are ideal carriers for delivering bay leaves extract topically. This study aims to investigate the potential of a bay leaves-containing hydrogel as a novel treatment for dermatitis. Bay leaves also called as *Laurus nobilis* belongs to family *Lauraceae* contain chemical compounds such as Eugenol, Anthrocyanin, Linalool, β-caryophyllene, Quercetin, and Rutin, which have been studied for their potential therapeutic effects in dermatitis. Bay leaves contain a unique phytonutrient, called parthenolide, that can quickly reduce inflammation and irritation when applied topically to affected area.

Mechanism
The mechanism of hydrogel on the skin involves several key processes that contribute to its beneficial effects:

1. **Moisture retention:** Hydrogels are highly absorbent materials that can hold a significant amount of water. This is particularly useful for individuals with dry or dehydrated skin, as it helps to restore moisture levels and improve skin texture.
2. **Barrier function:** Hydrogels can form a protective barrier on the surface of the skin, which helps to prevent moisture loss and protect against environmental irritants and pollutants. As it helps to maintain the skin's natural defences and promote healing.
3. **Cooling effect:** This cooling effect can help to soothe and relieve discomfort associated with sunburn, insect bites, or other forms of skin irritation.
4. **Drug delivery:** Hydrogels can serve as effective carriers for delivering drugs, vitamins, or other active ingredients to the skin. The porous structure of hydrogels allows for the controlled release of these substances, which can penetrate the skin barrier and target specific areas of concern.
5. **Biocompatibility:** This means that hydrogels are unlikely to cause irritation or allergic reactions when applied topically, making them suitable for a wide range of skincare and dermatological applications.

Advantages

The hydrogel containing bay leaves offers several advantages for dermatological applications:

1. **Natural Antimicrobial Activity:** Bay leaves contain phenolic compounds with known antibacterial properties, making them effective against pathogens such as Staphylococcus aureus. Incorporating bay leaves extract into hydrogel formulations provides a natural and potent antimicrobial effect, which can aid in the treatment of bacterial skin infections.
2. **Anti-inflammatory Properties:** Bay leaves are rich in bioactive compounds that possess anti-inflammatory properties. This help to reduce inflammation associated with dermatitis, eczema, and other skin conditions, providing relief to patients suffering from skin inflammation.
3. **Sustained Drug Release:** Hydrogels are known for their ability to provide sustained drug release, ensuring prolonged therapeutic effects. By encapsulating bay leaves extract within the hydrogel matrix, controlled release of bioactive compounds can be achieved, leading to prolonged action at the site of application.
4. **Moisturizing Effect:** Hydrogels have high water content and can help maintain skin hydration. It enhances the moisturizing effect of the gel, promoting skin hydration and barrier function, which is essential for maintaining healthy skin.
5. **Biocompatibility and Safety:** Hydrogels are generally well-tolerated by the skin due to their biocompatible nature. This formulation is less likely to cause skin irritation or adverse reactions, making them suitable for long-term use.
6. **Ease of Application:** Hydrogel formulations are easy to apply and spread evenly over the skin. They can be formulated into various forms such as gels, creams, or patches, providing flexibility in application and convenience for patients.

Overall, the hydrogel containing bay leaves extract offers a natural, effective, and safe option for the management of various dermatological conditions, with potential benefits including antimicrobial activity, anti-inflammatory effects, sustained drug release, moisturizing properties, biocompatibility, and ease of application.

Materials and Methods

Material: The leaves of *laurus Nobili's* obtained from authenticated crude drug store. Isopropyl myristate, Liquid paraffin, glycerine, methyl paraben obtained from Loba chemical. Guar gum and aloe vera obtain from Sigma Chemicals.

Collection of plant material

The leaves of *laurus Nobili's,* were collected from different matured plant and Store in air tight bottle for study.

Preparation of Bay leaves extract

Collected dried bay leaves were converted into a fine powdered and then extracted. The extraction process for Bay leaves involves two main methods: maceration and percolation. During maceration, coarsely powdered bay leaves are placed in a container and covered with a menstruum, which is left to soak for at least 4 hours. Subsequently, the macerated material undergoes percolation, a conventional extraction technique where the powdered material is added to a percolation tank and ethanol solvent is continuously added, allowing for the simultaneous collection of the percolation extract. In this process, 100 grams of powdered bay leaves are mixed with ethanol solvent in the percolator.

Formulation of hydrogel

In a heat-resistant container, add the distilled water and begin heating it gently using a water bath or a double boiler setup. Sprinkle the gelling agent slowly and evenly into the heated water while stirring continuously. This step is crucial to avoid clumping. Continue stirring until the gelling agent is fully dispersed, and the mixture becomes smooth and thickens into a gel-like consistency. This process might take some time; Once the hydrogel is formed, remove it from the heat source and allow it to cool to room temperature. Add the glycerine, aloe Vera gel, preservative, emollients, and antioxidant to the hydrogel base. Stir well after adding each ingredient to ensure proper incorporation. Once all the ingredients are thoroughly mixed, transfer the hydrogel cream into clean, airtight containers.

Formulation Table

Table 1: Content of Different formulations.

	Ingredients	F1	F2	F3	Use
1	Bay leave Extract (API)	2 ml	4 ml	6 ml	Active ingredient
2	Isopropyl myristate	1 ml	1 ml	1 ml	Penetration enhancer
3	Guar gum	1 gm	2 gm	5 gm	Gelling agent
4	Aloe vera	5 ml	10 ml	12 ml	Moisturizing agent
5	Liquid paraffin	5 ml	5 ml	5 ml	Emollient
6	Glycerine	5 ml	5 ml	5 ml	Humectant
7	Methyl paraben	1 gm	2 gm	2 gm	Preservative
8	Dist. Water	q.s ml	q.s ml	q.s ml	Q.S

Evaluation of hydrogel

Physical characterization: Obtain extract was characterized for its physical appearance with reaspect to its colour, odour and homogeneity. Marketed Hydrogel was taken as reference.

The characterization of the extract reveals that after the extraction process, the solution obtained from Bay leaves exhibits a dark green color. The pH of the solution falls within an ideal range of 6-7, although the plant demonstrates versatility and can tolerate pH levels ranging from 4.5 to 7.4. Regarding odor, Laurus Nobili's bay leaves are characterized by a pungent aroma with a sharp, bitter taste, while their dried form emits an herbal fragrance with subtle floral undertones. Additionally, the solubility of the extract indicates that it is soluble in both water and alcohol.

pH of formulation
Samples of formulation were taken in a test tube, then we a strip of litmus paper diped into the sample for a few seconds and wait for the indicator bars on the paper to change color. Compare the end of the test strip with the color chart that came with the paper to establish the pH level of the liquid.pH fo hydrogel was also recorded using ph meter where 1% of hydrogel Mixed with distilled water and pH was Recorded.

Spreadability
Excess sample was placed between the two glass slides and 100 g weight was placed on the glass slide for 5 min to compress the sample to a uniform thickness. Weight (250 g) was added to the pan. The time in seconds required to separate the two slides was taken as a measure of spreadability.

$S = m * l/t$

M – weight tied on upper slide, L – length of glass slide, T – time in sec

Viscosity: The prepared solution is placed in the Brookfield Viscometer and a proper speed is selected for the spindle according to the expected viscosity of the sample. The Brookfield Viscometer determines viscosity by measuring the force to turn the spindle in the solution at a given rate.

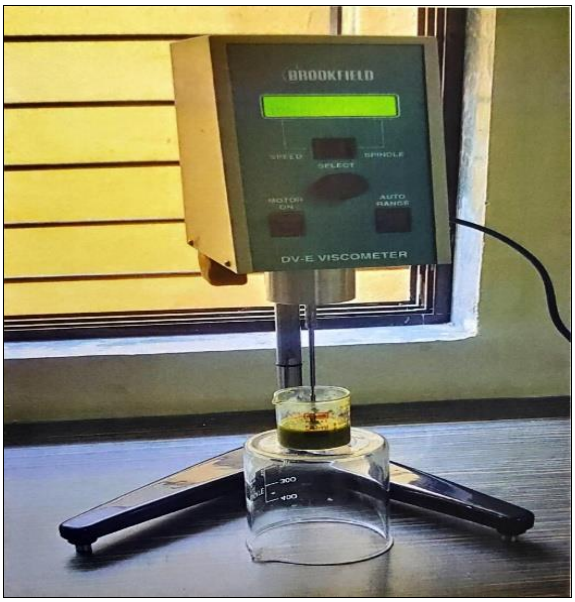

Fig 1: Viscosity determination by Brookfield Viscometer

Thin Layer Chromatography (TLC)
Thin Layer Chromatography (TLC) was employed to analyze the composition of the hydrogel containing bay leaves extract. TLC is a chromatography technique used to separate components in mixtures. In this method, the sample is spotted onto a TLC plate, which is then placed in a solvent chamber. As the solvent migrates up the plate via capillary action, different compounds in the sample move at different rates based on their affinity for the stationary and mobile phases. Visualization is typically achieved using UV light or staining agents. TLC revealed the presence and relative distribution of various chemical constituents within the hydrogel formulation, aiding in its characterization and quality assessment.

TLC Plate 1
In the chromatographic analysis utilizing TLC, the mobile phase consisted of a 1:1 mixture of ethyl acetate and ethyl ether, while the stationary phase was a solution of caffeine prepared by dissolving caffeine powder in ethanol. The Rf values, calculated as the ratio of the distance traveled by the solute to the distance traveled by the solvent, were determined for both standard and sample compounds.
In TLC Plate 2, the chromatographic separation employed a mobile phase composed of a mixture of sulphuric acid and methanol in a 9:1 ratio, while the stationary phase consisted of a solution of caffeine prepared by dissolving caffeine powder in ethanol.

UV-VIS Spectroscopy
Different dilutions of Bay leaf extract was prepared with phosphate buffer ph6.8 and was scan between 800-200nm wavelength for recording the Absorbance Maxima. After Taking Absorbance maxima dilutions of extract from 2-12µg/ml. Absorbance was recorded and plotted against concentration to get calibration curve. Similarly, for Hydrogel formulation containing Bya leaf extract different dilutions were prepared and absorbance was recorded.

Antibacterial Activity
Antibacterial as well as antiviral activity of a molecule is completely associated with the compound that provincially kill bacteria and virus or slow down their rate of growth, without being extensively toxic to nearby tissues. Most recently discovered antimicrobial agents are modified natural compound and this modification is done through chemical mode.
Antibacterial activity of a molecule is closely associated with its ability to either kill bacteria inhibit their growth rate, while minimizing toxicity to surrounding tissues. Many recently discovered antimicrobial agents are derived from natural compounds and undergo chemical modifications to enhance their efficacy. In an experiment, one milliliter of Staphylococcal aureus bacterial suspension stock was transferred into a sterilized petri dish and mixed with nutrient agar medium. After homogenization, approximately 10 mg of each hydrogel sample was placed on the prepared wells. The petri dishes were then incubated for 24 hours at 37°C. The ability of the hydrogel samples to inhibit the growth of Staphylococcus aureus was assessed by measuring the diameter of inhibition zones.

Results
Physical characterization: The characterization of the extract reveals that after the extraction process, the solution obtained from Bay leaves exhibits a dark green color. The pH of the solution falls within an ideal range of 6-7, although the plant demonstrates versatility and can tolerate pH levels ranging from 4.5 to 7.4. Regarding odor, Laurus Nobili's bay leaves are characterized by a pungent aroma with a sharp, bitter taste, while their dried form emits an herbal fragrance with subtle floral undertones. Additionally, the solubility of the extract indicates that it is soluble in both water and alcohol.

pH
Responsive hydrogels have been developed, exhibiting the

highest swelling capacity in comparison to both acidic and basic mediums, along with selective and controlled drug delivery observed specifically at ph 6.4, distinguishing it from acidic and basic conditions.

Spread ability

Was determined using a specialized apparatus consisting of a wooden board with a scale and two glass slides equipped with pans on both sides, mounted on a pulley system. Excess sample was sandwiched between the glass slides, and a weight of 0.40 g was placed on the upper glass slide for 5 minutes to achieve uniform compression. The time taken in seconds for the two slides to separate was recorded as a measure of spread ability. The spread ability (S) was calculated using the formula S = (m * l) / t, where m represents the weight tied on the upper slide, l denotes the length of the glass slide, and t indicates the time in seconds. Substituting the values, the spread ability was determined to be 0.24.

Viscosity

A maximum of 100 g of gel was placed into a container and positioned on a Brookfield viscometer equipped with spindle number 64. The spindle was then lowered onto the gel at a speed of 10 rpm. The viscosity of the gel was measured within a range of 2,017.03 to 3,866.52 cps. The viscometer settings included a torque range of 4.1, a spindle number of 07, a speed of 100 RPM, and a pressure conservation factor of 1640. Specifically, for spindle 07, the factor number used for calculations was 400.

Thin Layer Chromatography (TLC)

TLC Plate 1

In the chromatographic analysis utilizing TLC, the mobile phase consisted of a 1:1 mixture of ethyl acetate and ethyl ether, while the stationary phase was a solution of caffeine prepared by dissolving caffeine powder in ethanol. The Rf values, calculated as the ratio of the distance traveled by the solute to the distance traveled by the solvent, were determined for both standard and sample compounds. The standard compound exhibited an Rf value of 0.30 cm, while the sample compounds showed Rf values of 0.67 cm and 1 cm, respectively. The distance traveled by the solvent during the experiment was measured at 5.5 cm.

TLC Plate 1

Mobile phase – Ethyl acetate: Ethyl ether (1:1)

Stationary phase – Caffeine solution (caffeine powder dissolve in ethanol)

$$\text{RF value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by Solvent}}$$

Table 2: Table indicating RF values of standard and sample solution

Standard	1.7 cm
Sample	3.7, 5.5 cm
Distance travelled by solvent	5.5 cm
Rf value of sample 1	0.67 cm
Rf value of sample 2	1 cm
Standard	0.30 cm

In TLC Plate 2, the chromatographic separation employed a mobile phase composed of a mixture of sulphuric acid and methanol in a 9:1 ratio, while the stationary phase consisted of a solution of caffeine prepared by dissolving caffeine powder in ethanol. The standard compound displayed an Rf value of 0.48 cm, while the sample compound exhibited an Rf value of 0.42 cm. The distance traveled by the solvent during the experiment was measured at 3.5 cm.

TLC Plate 2

Mobile phase – Sulphuric acid: methanol (9:1)

Stationary phase – Caffeine solution (caffeine powder dissolve in ethanol)

$$\text{RF value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by Solvent}}$$

Table 3: Table indicating RF values of standard and sample solution

Standard	1.7 cm
Sample	1.5 cm
Rf value of sample 1	0.42 cm
Standard	0.48 cm
Distance travelled by solvent	3.5 cm

Fig 2: TLC plate 2

In TLC Plate 3, the chromatographic analysis utilized a mobile phase comprising a mixture of hexane and chloroform in a 6:4 ratio, while the stationary phase consisted of a solution of caffeine prepared by dissolving caffeine powder in ethanol. The standard compound exhibited an Rf value of 0.3 cm, while the sample compounds displayed Rf values of 0.1 cm and 0.17 cm, respectively. The distance traveled by the solvent during the experiment was measured at 4 cm.

TLC Plate 3

Mobile phase – Hexane: Chloroform (6:4)

Stationary phase – Caffeine solution (caffeine powder dissolve in ethanol)

$$\text{RF value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by Solvent}}$$

Table 4: Table indicating RF values of standard and sample solution

Standard	1.2 cm
Sample	0.4 , 0.7 cm
Rf value of sample 1	0.1 cm
Rf value of sample 2	0.17 cm
Standard	0.3 cm
Distance travelled by solvent	4 cm

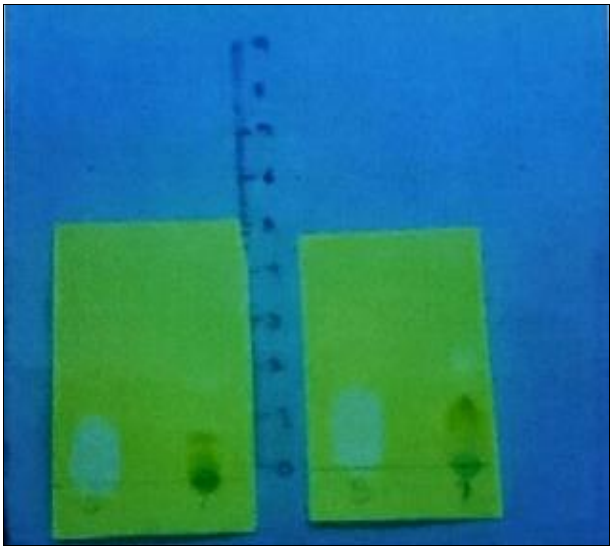

Fig 3: TLC plate

UV-VIS Spectroscopy

For drug content analysis, 1 gram of hydrogel was accurately weighed and dissolved in 80 ml of buffer solution at pH 6.8. The solution was sonicated for 10 minutes and then made up to a volume of 100 ml with phosphate buffer. Subsequently, 1 ml of this solution was pipetted out and diluted to 10 ml with the same solvent. The absorbance of the diluted solution was measured using UV spectroscopy at 279 nm against a blank.

The absorbance max of Bay leaves extract was determined to be 279 nm, while for the formulation, it was found to be 257 nm. These values enable the quantification of their concentrations in a sample by measuring the light absorbed at those wavelengths. A calibration curve, which illustrates the relationship between the concentration of a substance and its response, was generated for both the extract and formulation. The calibration curve for the extract exhibited an R^2 value of 0.9983, indicating a strong correlation between concentration and response.

Similarly, the calibration curve for the formulation showed an R^2 value of 0.9919, suggesting a good fit of the linear regression model. Dilution procedures were performed for the hydrogel formulation and extract to prepare samples for analysis. Absorbance measurements and calibration curves were obtained at 257 nm and 279 nm for the formulation and extract, respectively. Using the dilution factor method with a dilution ratio of 1:25, where 12.5 ml of the initial drug solution was mixed with 12.5 ml of buffer solution, the resulting solution was further diluted to 1 ppm. By applying the formula Y = mx + c (from the calibration curve), where Y is the absorbance, x Is the concentration, m is the slope, and c is the intercept, the concentration of the extract in 12.5 ml of formulation was calculated to be 36.19 mg/ml. Multiplying this concentration by the volume of the formulation (12.5 ml) yielded a drug content of 4.52 mg. Therefore, the drug content in the formulation was determined to be 4.52 mg.

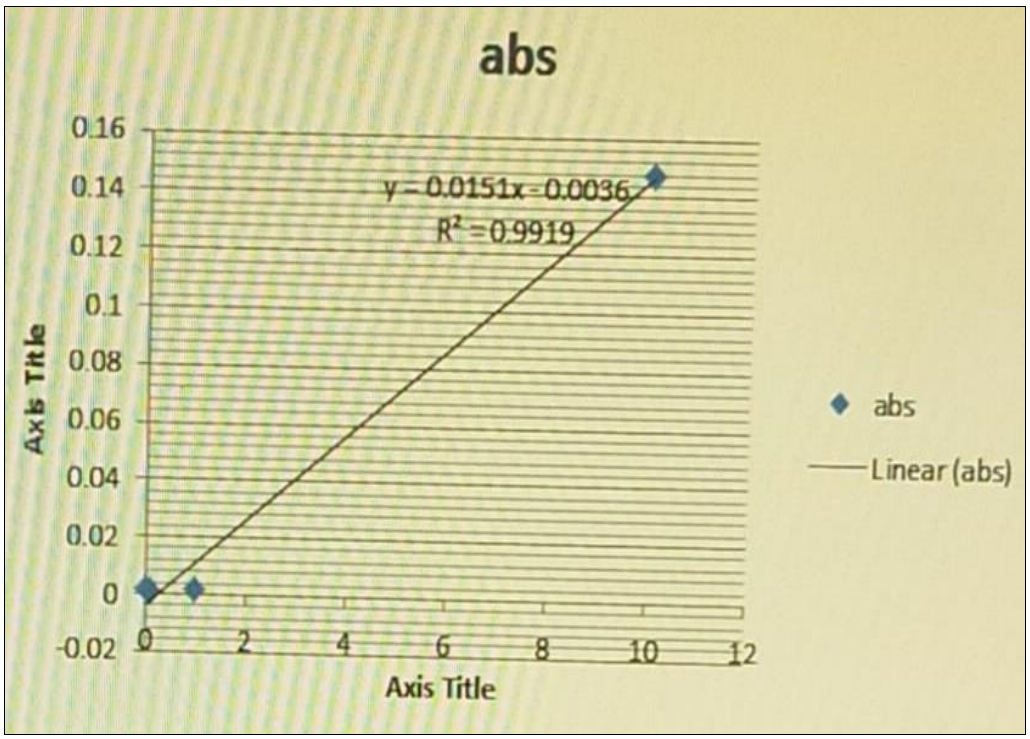

Fig 4: Calibration curve of Hydrogel containing Bay Leaf Extract.

Antibacterial activity

The extract exhibited a zone of inhibition is 1.5cm, while the known sample B showed a zone of inhibition of 2.5cm, and Standard 1 had a zone of inhibition of 2cm. The gel formulation displayed a zone of inhibition of 1.5cm. By applying the formula (Zone of inhibition of known sample / zone of inhibition of unknown sample) ÷ (Concentration of known sample / concentration of unknown sample), it was calculated that the concentration of the unknown sample was 30mm, considering a known standard concentration of 40mg. The results indicated that hydrogels prepared with different gelling agents derived from bay leaves extract exhibited similar activity against S. aureus. The antibacterial potency of bay leaves is attributed to their phenolic groups, which are known to possess antibacterial properties.

Negative controls consisting of hydrogel bases showed no inhibition zones, indicating a lack of antibacterial activity. In contrast, the positive control, which utilized gel, exhibited an inhibition zone diameter of approximately 30 mm. Among the hydrogel samples, the largest inhibition zone diameter was observed in the hydrogel containing solid dispersion as the gelling agent.

Overall, the positive control demonstrated inhibition comparable to that of synthetic products, highlighting the effectiveness of the hydrogel formulations in inhibiting the growth of S. aureus.

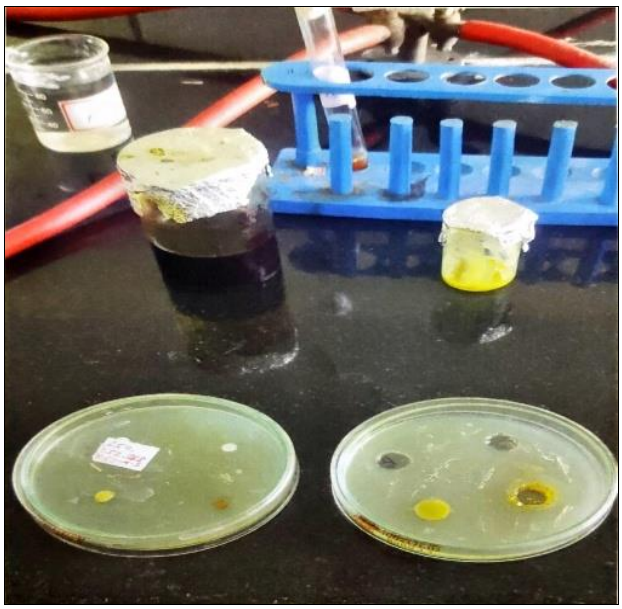

Fig 5: Antimicrobial activity of Bay Leaf Extract and hydrogel containing bay leaf Extract.

Conclusion

Formulation and evaluation of hydrogel formulations containing bay leaves extract demonstrate promising potential for dermatological applications. The incorporation of bay leaves extract into hydrogel matrices offers several advantages, including sustained drug release, enhanced permeability, and improved therapeutic efficacy against dermatitis. The physicochemical characterization of the hydrogels revealed their suitability for topical application, with favorable rheological properties and controlled drug release profiles.

Furthermore, the antimicrobial activity of the hydrogel formulations against Staphylococcus aureus underscores their potential in treating bacterial skin infections. The presence of phenolic compounds in bay leaves extract contributes to the observed antibacterial activity, providing a natural alternative to synthetic antimicrobial agents.

Overall, the results indicate that bay leaves-containing hydrogels hold promise as effective and safe therapeutic options for dermatitis management. Further studies, including clinical trials, are warranted to validate their efficacy and safety profiles in clinical settings and to explore their potential for commercialization as novel dermatological formulations.

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