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Preparation and Assessment of a Topical In-Situ Gel incorporating Aloe Vera Extract as a Promising Antibacterial

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Abstract

Aloe Vera has gained significant popularity in both traditional and official medicinal practices. Traditionally, the gel produced by the plant has been used to heal burns and wounds, among other conditions. The formulation and characterisation of an in-situ gel formulation containing aloe vera extract for topical application are the primary objectives of the current study. Carbopol 934 (CP934), hydroxy propyl methyl cellulose (HPMC), and Pluronic F127 (Pl-F127) were all employed in the cold method formulation of the in-situ gel. A total of eight distinct formulations were created, each varying in the ratio and type of polymers that form the hydrogel. The evaluations of all formulations included the analysis of their appearance, consistency, homogeneity, transparency, and the temperature at which gelation occurs. Based on the established evaluation parameters, the ideal formulations underwent additional assessment concerning pH measurement, spreadability, viscosity, gel strength, gelling capacity, skin irritation tests, and in-vitro antibacterial assay. The evaluation study's findings suggest that the AVg1 formula is a good herbal alternative for treating bacterial infections that occur topically.

Keywords: Aloe Vera, In-Situ Gel, Wound healing, Physicochemical properties, Antibacterial assay.

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إعداد وتقييم جل موضعي يحتوي على مستخلص الصبار كمضاد للبكتيريا واعد

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الملخص

اكتسب الصبار شعبية كبيرة في كل من الممارسات الطبية التقليدية والرسمية. تقليديا، تم استخدام الجل الذي ينتجه النبات لعلاج الحروق والجروح، من بين حالات أخرى. صياغة ووصف تركيبة هلام موضعي تحتوي على مستخلص الصبار للتطبيق الموضعي هي الأهداف الرئيسية للدراسة الحالية. تم استخدام Carbopol 934 (CP934)وهيدروكسي بروبيل ميثيل السليلوز (HPMC) و-Pluronic F127 (Pl-F127)في صياغة الطريقة الباردة للهلام الموضعي. تم إنشاء ما مجموعه ثماني تركيبات مميزة، تختلف كل منها في نسبة ونوع البوليمرات التي تشكل الهيدروجيل. تضمنت تقييمات جميع التركيبات تحليل مظهرها وقوامها وتجانسها وشفافيتها ودرجة الحرارة التي يحدث عندها التجلط. بناءً على معايير التقييم المحددة، خضعت التركيبات المثالية لتقييم إضافي فيما يتعلق بقياس الرقم الهيدروجيني وقابلية الانتشار واللزوجة وقوة الهلام وقدرته على التجلط واختبارات تهيج الجلد واختبار مضاد للبكتيريا في المختبر. تشير نتائج دراسة التقييم إلى أن تركيبة AVg1 تُعدّ بديلاً عشبياً جيداً لعلاج الالتهابات البكتيرية الموضعية.

الكلمات المفتاحية: الصبار، جل موضعي، التئام الجروح، الخصائص الفيزيائية والكيميائية، اختبار مضاد للبكتيريا.

Introduction

The human immune system relies heavily on the skin, which serves as the body's first line of defense and protects internal organs from the outside environment (Siang et al., 2014). Burns are classified among the most severe skin injuries and are a prominent public health crisis that affects populations worldwide. The surface of a burn is exposed to bacterial invasion after injuries, which can result in consequences like inflammation, sepsis, and other infections (Siang et al., 2014). The elevated bacterial count in the wound region can hinder the effectiveness of growth factors

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that are necessary for accelerating wound healing. In spite of the recent progress made in antimicrobial chemotherapy, infections remain a significant challenge in the treatment of burn injuries (Hamman, 2008). Current findings suggest that some agents that were previously effective are no longer successful in inhibiting bacterial growth (Barkat et al., 2017; Shahzad & Ahmed, 2013). The regeneration of skin is impacted by various factors, notably the preservation of moisture in the wound area, efficient oxygen flow, and a minimal bacterial load (Al-Bazzaz & Ismail, 2024; Barkat et al., 2017).

Within the diverse categories of wound dressings, hydrogel-based options are receiving heightened attention from both researchers and market stakeholders (Barkat et al., 2017). The term "in situ," derived from Latin, refers to the state of being administered directly within the body, whereupon administration, the substances undergo a gelation process to create a gel (Chaudhary & Verma, 2014). The thermosensitive properties of Pluronic F127 (Pl-F127) facilitate the promising application of its micelles in drug delivery systems (Dantas Silva et al., 2018). Carbopol and Hydroxypropyl methylcellulose (HPMC) are frequently recommended for use in drug formulation and delivery owing to its desirable properties (Al-Bazzaz & Ismail, 2024; Siang et al., 2014).

One of the oldest natural therapies for improving skin health is aloe vera (AV) (Paulsen et al., 2005; Saleem et al., 2022). According to studies, AV improves the mechanical features and biocompatibility of hydrogel-based matrices and films when combined with other polymers. The high water-absorbing capacity of AV, along with its ease of modification and grafting, makes it a preferred choice over other polysaccharides (Ajaz et al., 2023). The antifungal activity, anti-inflammatory properties, hypoglycemic effects, anti-obesity techniques, immunomodulatory functions, anticancer effects, and treatment options for burns, insect stings, skin inflammation, psoriasis, and genital herpes are just a few of the many health advantages linked to AV. In addition to its analgesic, antibacterial, and antiviral activities, AV is known for its antiseptic properties, cardioprotective effects, and protective advantages for skin and bone. It is also widely used in cosmetic applications (Ashouri et al., 2019; Dadashzadeh et al., 2020; Haasbroek et al., 2019; Kurian et al., 2018; Sabzaligol, M., Safari, N., Baghcjeghi, N., Latifi, M., Bekhradi, R., Taghizadeh, M., & Zareie, 2014; Saleem et al., 2022; Yahya et al., 2022).

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AV shows great promise in maintaining the integrity of the skin, lowering erythema, and preventing skin ulcers (Saleem et al., 2022), as well as for alleviating swelling and pain that arise from wounds and burns (Dadashzadeh et al., 2020). The effectiveness of AV in wound healing can be attributed to several mechanisms, which encompass the preservation of moist environments, enhancement of cellular migration, stimulation of collagen synthesis, facilitation of tissue regeneration, minimization of scarring, and attenuation of inflammatory responses (Dadashzadeh et al., 2020; Saleem et al., 2022; Ul-Islam et al., 2021). Both Gram-positive and Gram-negative bacteria are susceptible to its broad spectrum of activity (Al-Nima, 2021; Saleem et al., 2022). In Denmark, the Danish Medicines Agency has authorized a preserved, yet unmodified AV gel as a topical herbal treatment in Denmark, specifically for addressing minor wounds, burns, sunburn, and frostbite (Paulsen et al., 2005). The present work aims to prepare an Aloe Vera extract and a topical in-situ gel that incorporates this extract. Furthermore, it aims to assess the physical characteristics of the gel as well as its antimicrobial efficacy, reflecting a shift towards plant-based treatments, which are frequently viewed as safer and associated with a lower incidence of side effects compared to synthetic alternatives.

Methodology

Materials

Fresh AV leaves were collected from Local gardens, Al Marj, Libya. The materials used in this study were Pluronic® F127 (Pl-F127) was obtained from El-Amryia Pharmaceutical Ind. Co., Alexandria, Egypt and Hydroxypropyl methylcellulose (HPMC) K4m (4000 cP) and Carbopol® 934 (CP934) were obtained from El Pharaonia Pharmaceutical Co. (Alexandria, Egypt). The other reagents were all analytical grade.

Equipments

Sensitive electronic balance (ME235S, SARTORIUS AG, Germany), Brookfield digital viscometer (DV-II, Brookfield Engineering Laboratories, INC., USA), Hotplate & Magnetic stirrer (JENWAY 1000,UK), Digital pH meter (S220 Seven Compact™ pH/Ion, Mettler Toledo Co., Switzerland), Electric blender (Royal-Japan), Advanced IR vortex mixer (VELP SCIENTIFICA, Italy), Incubator (Thermoscientific Heraeus, T12, InnerTekna, Germany), Oven (Thermoscientific Heraeus, UT6,

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InnerTekna, Germany), Home Mill (BLACK& DECKER, 02, England), Rotary Evaporator (Buchi, Model 462, Germany), Orbital Shaker (Heidolph Instruments, Schwabach, Germany).

Methods

Preparation of AV extract

AV leaves were ground into a powder after being dried for 24 hours at 70°C in an oven. Around two grams of AV powder were mixed with 10 ml of 96% ethanol and placed on a shaker for 72 hours to ensure complete dissolution. After filtering the mixture, the filtrate was concentrated using a rotary evaporator to produce dry extract. To be used later, it was kept in a refrigerator at 4°C (Tinggi et al., 2022).

Trials for the selection of optimal gelling agent

Several in-situ gel formulations (AVg1-AVg8) were developed with varying concentrations of Pl-F127, with or without the addition of HPMC and/or CP934, to ascertain the best option, as detailed in Table (1). The best option was ultimately determined to be the in-situ gel formulation, which stays liquid at room temperature and turns into a gel at body temperature (37°C). The AV extract was incorporated into this formulation for additional assessment.

In-situ gel preparation

A cold method utilizing Pl-F127, HPMC, and/or CP934 was used to create a number of placebo formulations of thermosensitive in-situ gels. In summary, CP934 and HPMC were slowly incorporated into vigorously stirred distilled water until the polymer molecules were fully hydrated at room temperature. In order to create a homogenous solution, Pl-F127 was then gradually added to this polymer dispersion while being constantly stirred in an ice-cooled water bath. To guarantee that the polymer molecules were fully hydrated, the resultant solution was thereafter placed in tightly sealed containers and kept at 4°C for 24 hours. To create the AV extract-containing in-situ gel (AVgext), the extract was added at the beginning of the preparation procedure, before the polymers were added.

Evaluation of in-situ gel

The optimal formula determination

Table (1) illustrates the preparation of eight formulations utilizing varying concentrations of three distinct polymers: Pl-F127, HPMC, and/or CP934, aimed at identifying the optimal gel formulations. The optimal formula was selected based on

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parameters like gel appearance, consistency and sol-gel temperature. Following the selection of the suitable gel formulas, AV extract (10%w/w) was included, and these formulations were subjected to further assessment.

Table.1. The thermosensitive in-situ gel formula's composition

Code	Pl-F127 (%w/w)	CP934 (%w/w)	HPMC (%w/w)	Distilled water q.s. to
AVg1	**15**	**0.12**	**0.6**	**100**
AVg2	**17**	**0.12**	**0.6**	**100**
AVg3	**18**	**-**	**0.5**	**100**
AVg4	**19**	**0.12**	**0.6**	**100**
AVg5	**20**	**1**	**-**	**100**
AVg6	**20**	**-**	**-**	**100**
AVg7	**20**	**0.4**	**-**	**100**
AVg8	**20**	**-**	**0.4**	**100**

Homogeneity and transparency

Test for homogeneity was achieved visually after the gel had properly settled in a beaker. The formulae were examined in terms of transparency, gel removal effectiveness, after feel, and smear type (Al-Nima, 2021).

Appearance and consistency

The prepared formulations underwent visual inspection to determine their appearance and consistency after being filled into a suitable container, with assessments performed on contrasting white and dark backgrounds. Examinations have been conducted with respect to their color, appearance in gel form, and the presence of aggregates (Al-Nima, 2021).

Gelation temperature determination (T sol-gel)

A test tube containing two milliliters of solution was dipped in a water bath that was kept at 37 °C. A thermometer was inserted into the test tube to determine the temperature at which the solution turned into a gel. It was considered that the gel developed in case the formulation remained in place when the test tube was upside-down (Chaudhary & Verma, 2014).

Determination of Gelling Capacity

It was assessed by calculating the time required for the gel to form. To test the gel's gelling ability, a drop of the gel was added to a beaker of water that was kept at 37 °C. The gelling time was then visually examined. The following grades were assigned: +; the gel dissolves quickly after a few minutes, ++; the immediate gelation

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lasts for a short while, and +++; the immediate gelation lasts for almost an hour (Aksu et al., 2019).

pH Assessment

A calibrated pH meter was used to measure the pH. Three separate determinations were made, and the average of these was calculated (Chaudhary & Verma, 2014).

Viscosity Measurement

A Brookfield viscometer with spindle number 4 was used to measure the viscosity at 25°C and 37°C. Shear rates of 5, 10, 20, 50, and 100 s^{-1} were performed. First, the gel solution's viscosity was measured. A water bath kept at 37 °C was then used to raise the solution's temperature and turn it into a gel. The resulting gel's viscosity was then measured, and the average of the three readings was determined (Chaudhary & Verma, 2014).

Spreadability Test

The mixture was heated in a water bath kept at 37 °C to turn it into a gel. A 20 cm × 20 cm glass plate was then placed with around 1 g of the gel in the middle. After then, a second glass plate with the same measurements was placed over this one. The gel spread between the plates when a 1000 g weight was carefully put on the top plate. The weight was taken off after a minute, and the spread area's diameter was measured in cm. Three duplicates of this experiment were carried out (Aksu et al., 2019; Chaudhary & Verma, 2014).

Gel Strength

A 50-ml graduated measuring cylinder was filled with a 30-ml sample of the solution, which was then left to gel in a water bath that was kept at 37°C. A second cylinder was used to apply a 50 g weight to the gel. The time (in seconds) it took for the cylinder to move 5 cm through the gel was used to calculate the gel's strength. Additional weights were placed on top of the cylinder in cases where it took longer than five minutes for it to submerge in the gel. The minimal weights needed to drive the cylinder through the gel by 5 cm was used to gauge the gel's strength. This assessment was performed in triplicate (Chaudhary & Verma, 2014; Galgatte et al., 2014).

Skin irritation test

This evaluation aimed to establish the optimal formulations that validate the gel's compatibility with the skin. It was tested on human volunteers to assess any irritancy issues that could hinder the gel's usability. Five individuals were selected to undergo the

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skin irritancy test. The formula under examination was applied topically to an area of nearly 2 square inches on the hand. Observations for redness, lesions, irritation, edema, and any signs of skin irritancy were conducted at regular intervals over a period of approximately 24 hours and documented (Al-Nima et al., 2020).

In-vitro antimicrobial assay

Assessment of antimicrobial efficacy in vitro

Both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria were acquired from the microbiology lab of the Omar Al-Mukhtar University College of Pharmacy. The disc diffusion method was used to evaluate the ideal formulations' antibacterial efficacy.

The inoculation plates were then incubated aerobically for 24 hours at 37˚C after the isolated bacteria had been subcultured on a selective medium tailored to each type of bacteria.

Producing standard concentrations of AV in-situ gel extract formulas and bacteria inoculation

A formula for AV in-situ gel extract was produced at a concentration of 100 mg/ml. Each bacterial type's pure cultures were separated as individual colonies and cultivated in Trypticase Soy Broth (TSB), which was then incubated for 24 hours at 37°C. The concentration was then adjusted with sterile TSB to 0.5 McFarland turbidity (Adzitey et al., 2019).

Susceptibility test

A sterile cotton swab was used to wipe isolates in TSB at a McFarland turbidity of 0.5 onto Mueller-Hinton (MH) agar.

Antibacterial assay

Filter paper was cut into small discs, which were then soaked in the AV in-situ gel formulation solution and control agents (SSD cream and Mebo cream). Following that, these discs were put on top of MH agar that had already been infected with the isolates. The antibacterial action was then evaluated by measuring the inhibition zones in millimeters (mm) during a 24-hour incubation period at 37°C. In order to avoid inhibition zone overlap, each disk was placed with sufficient distance between them (Adzitey et al., 2019).

Results and discussion

Homogeneity and transparency

Table (3) displayed the results of the homogeneity analysis of all developed formulations. Each of the prepared formulas

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demonstrated uniformity, providing a pleasant after-feel and exhibiting no signs of phase separation. The in-situ gel formulations AVg1, AVg2, AVg3, AVg6, and AVg8 exhibited greater homogeneity compared to all other prepared formulations. In contrast, the homogeneity of AVg4 and AVg7 gel formulations was deemed satisfactory. AVg5 gel formulation displayed limited homogeneity, which may be attributed to the elevated concentration of CP934 (1% w/w). Upon applying formulations topically, it was noted that all formulas were non-greasy and could be easily removed, except for AVg5 and AVg7, which contained 1% and 0.4% w/w CP934, respectively, along with 20% w/w Pl-F127. These formulations were found to be sticky and difficult to remove, possibly due to the elevated levels of CP934. The formulations that included CP934 (AVg1, AVg2, and AVg4) were predominantly transparent, with the notable exception of two formulations (AVg5 and AVg7), which appeared opaque. Specifically, the formulations AVg1, AVg2, and AVg4, which contained 15%, 17%, and 19% w/w Pl-F127 respectively, along with equal proportions of HPMC and CP934 (0.6% and 0.12% w/w), were found to be more homogeneous than the other CP934 formulations, as depicted in figure (1)

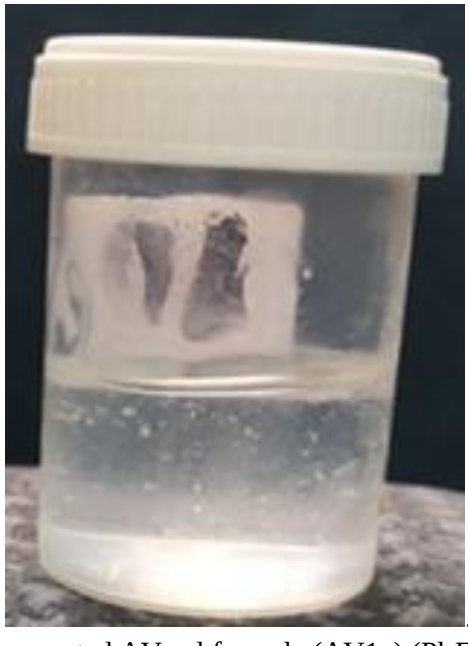

Figure 1. The accepted AV gel formula (AV1g) (Pl-F127 15%w/w, HPMC 0.6 %w/w and CP934 0.12%w/w)

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Appearance and consistency

The appearance and consistency are critical attributes of gel formulations. All the prepared formulas exhibited a smooth texture, free from aggregates or clumping, and were colorless. The formula AVg6 appeared thin, attributed solely to the inclusion of 20% w/w Pl-F127. In contrast, the formulas AVg5 and AVg7 exhibited thick consistency. The other formulas, AVg3, AVg4, and AVg8, demonstrated satisfactory consistency. All observations regarding AV gel are presented in Table (2).

Gelation temperature

T sol-gel was determined to be between 23°C and 37°C for all formulations. Formulations AVg5 and AVg7 formed gel immediately upon preparation, resulting in their exclusion from further characterization. The transition temperature of AVg1 was higher than that of the other formulations and matched the physiological temperature of the skin at 37°C. Thus, AVg1 was designated as the optimal formula for subsequent evaluation. Figure (2) showed the optimum formula: (a) at solution state (25 °C) and (b) at gel state (37°C).

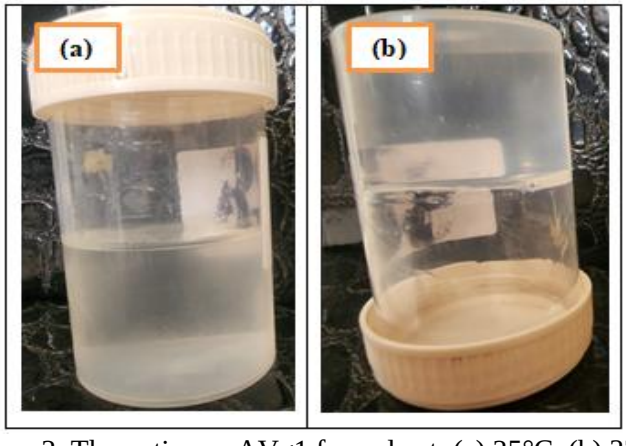

Figure 2. The optimum AVg1 formula at: (a) 25°C, (b) 37°C

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Table.2. Evaluation data 1 of AV in-situ gel formulas

+++ Excellent, ++ Good, + Satisfactory, - Bad

Parameter	AVg1	AVg2	AVg3	AVg4	AVg5	AVg6	AVg7	AVg8
Homogeneity	++	++	++	++	+	++	++	++
Transparency	Transparent	Transparent	Transparent	Transparent	Opaque	Transparent	Opaque	Transparent
Gel appearance	Excellent	Excellent	Very good	Good	Very thick	Thin	Thick	Very good
Gel consistency	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Color	No	No	No	No	No	No	No	No
Odor	No	No	No	No	No	No	No	No
Phase separation	No	No	No	No	No	No	No	No
After feel	Good	Good	Good	Good	Poor	Good	Good	Good
Type of smear	Non-greasy	Non-greasy	Non-greasy	Non-greasy	Sticky	Non-greasy	Sticky	Non-greasy
Removal	Easy	Easy	Easy	Easy	Not easy	Easy	Not easy	Easy

Table.3. Evaluation data 2 of in-situ gel formulations.

Code (Blank formula)	Gelation temperature (°C)
AVg1	**37**
AVg2	**27**
AVg3	**29**
AVg4	**23**
AVg5	**Gel formation immediately**
AVg6	**30**
AVg7	**Gel formation immediately**
AVg8	**27**

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The optimal formula determination

The present study examined how the type and concentration of gelling agents influenced the formulations created. As presented in Table (2), different types and concentrations of polymers were tested individually and in combination to identify the optimal formulations. The final formula was chosen based on several criteria, including homogeneity, transparency, consistency and Gelation temperature. Variations in physical parameters among the formulas were noted. The formulation (AVg6) that included 20% w/w Pl-F127 exhibited a very low viscosity, with gelation temperature of 30°C. However, this gel demonstrated insufficient mechanical strength and experienced rapid erosion. As the extended presence of drug formulations on the skin is essential for successful topical drug delivery, this formula was eliminated from further testing, and HPMC and CP934 were incorporated to resolve this drawback. Regarding concentration of gelling agent, the formulations AVg5 (which included 20% w/w Pl-F127 and 1% w/w CP934) and AVg7 (which contained 20% w/w Pl-F127 and 0.4% w/w CP934) yielded excessively thick and sticky mixtures that could not be adequately spread or even moved within the container, leading to their exclusion as well.

New batches were developed using HPMC. The formulations, AVg3 (which includes 18% w/w Pl-F127 and 0.5% w/w HPMC) and AVg8 (which contains 20% w/w Pl-F127 and 0.4% w/w HPMC) demonstrated satisfactory properties; however, they exhibited low consistency, with gelation temperatures of 29 and 27 °C, respectively. As a result, these formulations were not considered for further characterization. In contrast, the remaining formulations, AVg1, AVg2, and AVg4, which contained 15%, 17%, and 19% w/w Pl-F127, respectively, along with consistent concentrations of CP934 (0.12% w/w) and HPMC (0.6% w/w), proved to be excellent, with gelation temperatures of 37, 27, and 23 °C, respectively.

The formulations developed with Pl-F127, HPMC, and CP934 were favored over the other options. Considering all of the factors discussed earlier, formula AVg1 (comprising 15% w/w Pl-F127, 0.6% w/w HPMC, and 0.12% w/w CP934) demonstrated optimal performance. Subsequently, AV extract was incorporated into AVg1 formula for further assessment, which included pH, viscosity, spreadability, skin irritation testing, gelling capacity

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determination, gel strength evaluation, and potential antimicrobial activity.

Gelling Capacity

As demonstrated in Table (4), AVg1 formula exhibited immediate gelation lasting for approximately one hour. In contrast, AVg1ext formula demonstrated immediate gelation for about half an hour. This suggests that the inclusion of AV extract reduced the erosion time of the resulting gel.

pH results

Maintaining the pH level of any herbal gel is essential for both ensuring gel stability and reducing the possibility of skin irritation when the formula is applied topically. An appropriate pH range was offered by the chosen formulations, as shown in Table (4), which will aid to prevent local irritation. The pH values of AVg1 and AVg1ext were found to be 5.72 ± 0.01

Table.4. Evaluation data 3 of blank and-in situ gel formulas.

Formula	pH	Gelling Capacity	Gel Strength (seconds)	Spreadability (cm)
AVg1	**5.72 ± 0.01**	**+++**	**355 ± 0.50**	**8.0 ± 0.20**
AVg1$_{ext}$	**5.60 ± 0.02**	**++**	**340 ± 0.10**	**10 ± 0.03**

+: the gel dissolves quickly after a few minutes.
++: the immediate gelation lasts for a short.
+++: the immediate gelation lasts for almost an hour.
Mean ± S.D.; n = 3
AVg1: The blank formula for in-situ gel
AVg1$_{ext}$: Extract-containing in-situ gel

and 5.60 ± 0.02, respectively, both of which are within the recommended pH range for topical products (4.5 to 5.75). These formulations can therefore be used without risk of skin irritation. Additionally, it shows that the formulations' pH was not considerably affected by the substances used.

Viscosity outcomes

Viscosity is a vital attribute of the gel formulation, as it represents the gel's ability to resist flow when it comes into contact with the skin. Viscosity of semisolids should allow the ease of expression from the container and spreading on the lesion (Amasya et al., 2012).

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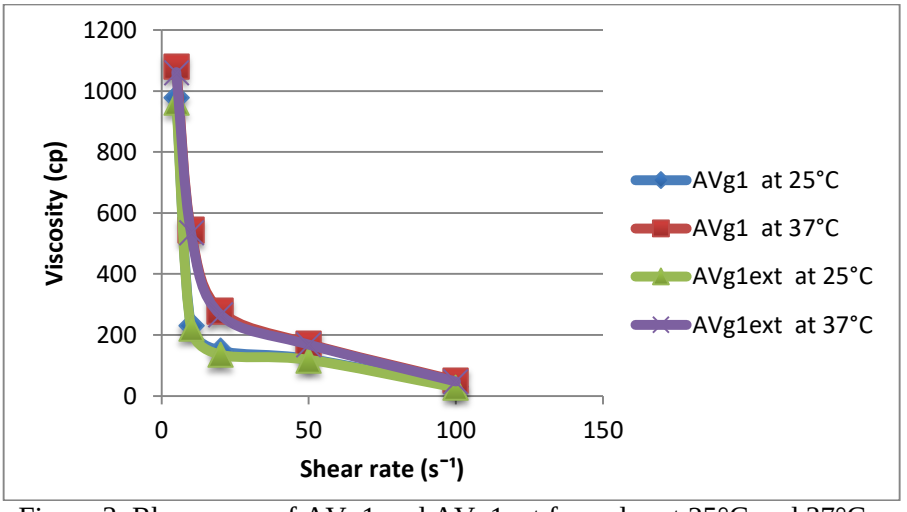

Figure 3. Rheograms of AVg1 and AVg1ext formulas at 25°C and 37°C

At 25°C, the viscosity of all formulations was comparatively low. But when the temperature was increased to 37°C, a gel formed, which caused the viscosity to noticeably increase. AVg1 formula showed a marginally higher viscosity compared to AVg1ext formula. The findings showed unequivocally that the viscosity values decreased as the shear rate increased which is apparent in Figure (3). Rheograms for both formulations reveal that all systems display non-Newtonian, pseudo-plastic (shear-thinning) behavior favorable for topical products (Dixit et al., 2011).

Spreadability results

A key criterion for topical formulations, particularly gel-based ones, is their ability to spread easily on the skin without any leakage post-application. Spreadability refers to the degree to which the formula can uniformly cover the skin or the targeted area upon application (Khan et al., 2013). The results illustrated in Table (4) indicated that, AVg1 and AVg1ext exhibited spreadability values of 8.0 ± 0.20 cm and 10 ± 0.03 cm, respectively. AVg1ext formulation demonstrated superior spreadability (10 ± 0.03 cm) compared to AVg1 formulation, attributed to its lower gel strength and viscosity. These spreadability findings align with the previously established viscosity data. For every formula in the current investigation, the spreadability results showed that the gel can be applied easily without running off and is spreadable with a minimal amount of shear.

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Gel Strength

Ensuring that the topical gel formulation has adequate gel strength is of utmost importance. An optimal in-situ gel should be designed to allow for easy application and to remain on the skin without leaking after being applied. Throughout this study, it was observed that all formulations took more than five minutes for the cylinder to be fully immersed in the gel. A 200 g weight was added to the cylinder to test the gel's strength, and the least amount of weight needed to press the cylinder down 5 cm into the gel was noted. The data regarding gel strength measurements is illustrated in Table (4). The gel strength for AVg1 was measured at 355 ± 0.50 seconds, while AVg1ext recorded a value of 340 ± 0.10 seconds. This indicates that AVg1 formulation has a slightly superior gel strength compared to AVg1ext, likely due to differences in viscosity between the two formulations.

Skin irritation results

The obtained results confirmed the absence of redness, edema, irritation, or any other negative reactions post-application. This finding supports the compatibility and safety of the optimal formula for skin application.

In-vitro anti-microbial assay results

In present study, the formulation AVg1ext was chosen for additional examination of its anti-microbial properties.

Table.5. The antibacterial activity of AVg1ext against E.coli and S. aureus

Microorganisms	AV g formula	Control	
	$AVg1_{ext}$	SSD	Mebo
	Inhibition Zone (mm)		
E.coli	**9**	**12**	**0**
S. aureus	**12**	**0**	**0**

The findings from the evaluation of the antibacterial effectiveness of AVg1ext formula effectiveness are presented in Table (5) and Figure (4). The outcomes indicated that AVg1ext formula exhibited antibacterial properties against both bacterial types, with a notably stronger effect observed against S. aureus. Inhibition zones of 9 mm for E. coli and 12 mm for S. aureus were shown by the AVg1text formula. In contrast, SSD control exhibited 12 mm inhibition zone against E. coli but showed no effect on S. aureus. Mebo control, on the other hand, did not produce any inhibitory effect on either bacterial strain.

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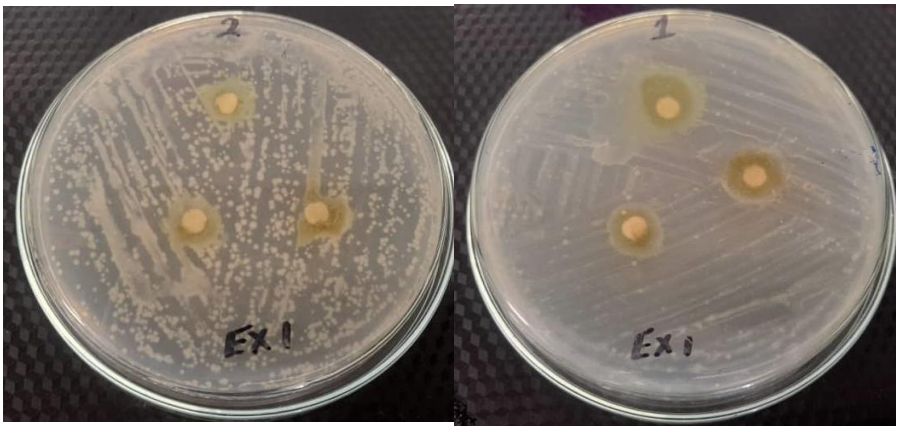
According to the study's findings, the produced AVg1ext had noticeably more antibacterial action against S. aureus than E. coli. V.C. Pawar et al.'s findings that AV gel extract totally stopped S. aureus growth are corroborated by their results. (Pawar et al., 2005). Furthermore, our findings align with those of Frederick et al., who conducted a comparison of various



E.coli *S.aureus*

Figure 4. The AVg1ext formula's antibacterial activity was a zone of inhibition against S. aureus and E. coli following a day of incubation at 37±2°C.

plant extracts, including AV, against several bacterial strains, including E. coli. It was reported that most bacterial species exhibited susceptibility to nearly all AV extracts when applied at high concentrations (Adzitey et al., 2016).

According to the findings of Al-Nima et al., AV gel developed showed a stronger antimicrobial response against S. aureus than against E. coli (Al-Nima, 2021). Frederick and his team achieved promising results in their 2016 study. It was found that AV gel demonstrates antibacterial activity against E. coli, utilizing disc agar diffusion method for their analysis (Adzitey et al., 2016). Darshan et al. provide evidence that the extract from the leaves of A. barbadensis demonstrates substantial antimicrobial activity against a diverse range of microorganisms (Dharajiya et al., 2017). The research by Kar and Bera et al. emphasizes that AV gel is rich in a range of secondary metabolites which are primarily responsible for its antimicrobial activity (Bera, 2018).

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Conclusion

A thermosensitive in-situ gel containing 10% w/w of AV was effectively developed using a cold method with Pl-F127, CP 934, and HPMC. The gelation temperatures across all formulations ranged from 23 to 37°C. This formulation aims to facilitate the effective use of this herb in both medical and cosmetic applications for users. Among the various formulations created, one was selected for additional research (AVg1). The chosen gel formulation demonstrated favorable physicochemical properties, such as consistency, satisfactory skin irritancy results, and an acceptable pH level. The chosen gel formulation also demonstrated strong antibacterial activity against E. coli and high levels of antimicrobial activity against S. aureus, the most frequent cause of skin infections. This indicates that the topical in-situ gel of AV can be produced with relative simplicity. Reliance on common antibiotics, which could lead to the emergence of resistance strains, may be reduced by using AV extracts as prophylactic antibiotics. The present study, in conclusion, emphasizes the importance of natural products in controlling bacteria that are resistant to antibiotics, which pose a serious threat to human health. Our findings indicate that the gel extract of AV can be utilized in the development of new drug formulations aimed at treating infectious diseases in humans.

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