RESEARCH PAPER

Hydrogel Formulation, Antimicrobial Action, and Cytotoxicity of Eurycoma longifolia Flower and Leaf Extracts Against ORL-48 Oral Cancer Cells

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ABSTRACT

Eurycoma longifolia Jack is a Southeast Asian medicinal plant traditionally used for various therapeutic purposes, yet its flowers and leaves remain understudied despite their potential bioactivity. This study aimed to extract, characterize, and evaluate methanolic extracts from the flowers and leaves of E. longifolia, and to incorporate these extracts into hydrogel formulations for potential wound healing applications. The flower extract produced a considerably higher yield than the leaf extract and contained measurable levels of phenolics and flavonoids. It also demonstrated strong antioxidant activity with an IC_{50} value of 11.604 ppm. Hydrogels formulated using alginate, PVP, and PEG exhibited acceptable physical and chemical characteristics, including appropriate viscosity, spreadability, water content, and pH values suitable for dermal application. Stability assessments showed that all formulations maintained colour and structural integrity for 21 days. Antimicrobial evaluation revealed strong inhibitory activity against Escherichia coli, Staphylococcus aureus, and Candida albicans across all hydrogel formulations. A multi attribute selection method identified the alginate:PEG (75:25) hydrogel containing leaf extract as the optimal formulation based on its balanced physicochemical properties and antimicrobial performance. In vitro scratch assays conducted on ORL-48 cancer cells showed that both flower and leaf extracts inhibited cell migration and proliferation, indicating cytotoxic effects. These results support the feasibility of incorporating E. longifolia extracts into hydrogel systems while highlighting the need for further studies using non-cancerous cell models to assess true wound healing potential.

Keywords: Eurycoma longifolia, Wound healing hydrogel, Antioxidant activity, Antimicrobial properties, Orl-48 cancer cell.

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1. INTRODUCTION

Eurycoma longifolia Jack is a well-known tropical medicinal plant that grows widely throughout mainland Southeast Asia and the Indonesian archipelago [1]. It is recognized by various vernacular names, including Tongkat Ali or Malaysian ginseng in Malaysia, Pasak Bumi in Indonesia, Ian-don in Thailand, Tho Nan in Laos, Cay Ba Benh in Vietnam, and Plaa-lai-pueak in Myanmar [2]. For centuries, this species has been used as a fundamental ingredient in traditional herbal preparations and is valued for its diverse therapeutic properties (Figure 1) [1].

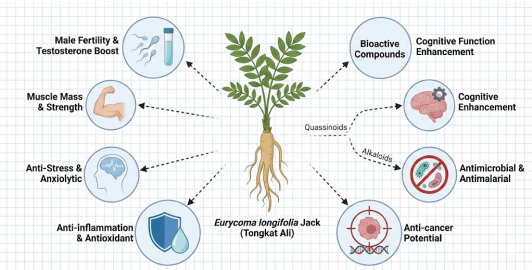

Figure 1: Therapeutic properties of *E. longifolia* Jack

Phytochemical investigations have revealed that *E. longifolia* contains a remarkably rich and complex profile of bioactive metabolites. More than 65 compounds have been identified, which include glycoproteins, saponins, and a variety of quassinoids such as quassin, neoquassin, glaucarubin, sedrin, eurycomanol, eurycomanol-2-O-β-D-glucopyranoside, eurycomanone, and 13β,18-dihydroeurycomanol [3]. Additional constituents such as eurikomalactone, eurikomanol, laurikomalactone A and B, dehydrocomalactone, non-benzoquinones, saponins, and sterol esters including sitosterol and stigmasterol have also been reported. Collectively, these chemical components contribute to the plant's well-documented pharmacological roles, which include antimicrobial, anti-inflammatory, and aphrodisiac effects [2].

Although the roots, stems, and bark of *E. longifolia* have been studied extensively, the flowers remain among the least explored parts of the plant. The floral structures consist of small petals arranged in 5 to 6 lobes and occur as either hermaphroditic or male forms. These flowers develop in large panicles, yet they have received very limited scientific attention [4]. Previous studies indicate that the flowers contain secondary metabolites similar to other plant parts, including alkaloids, saponins, terpenoids, steroids, and flavonoids [5, 6]. This suggests that the flowers may represent an underutilized source of bioactive compounds with significant therapeutic potential.

Wounds are defined as disruptions in the structure and function of tissues that result in the loss or damage of cellular components [7]. These injuries may be caused by physical, chemical, thermal, or pathological factors. The extent of tissue damage can vary greatly and may involve superficial epithelial layers or deeper tissues such as the subcutaneous layer, muscles, nerves, blood vessels, and bone [8]. Wound healing is a complex physiological process that can be impaired by bacterial contamination. Delayed treatment and inadequate protection of the wound increase the risk of infection, inflammation, and complications that may lead to poor healing outcomes.

Hydrogels have become an important class of wound dressing materials because of their hydrophilic nature, biocompatibility, and ability to create a moist environment that supports tissue repair [9]. Structurally, hydrogels are three-dimensional networks of polymer chains that absorb and retain significant amounts of aqueous fluid while maintaining their integrity [10]. They have been developed for various biomedical applications, including controlled drug delivery, contact lenses [11], enzyme and cell immobilization [12], and modern wound management systems [13]. The incorporation of natural extracts into hydrogel matrices is a promising strategy for enhancing both the functional and therapeutic properties of wound dressings.

Previous research has shown that extracts of *E. longifolia* possess strong antimicrobial activity. Kuspradini, Silau [14] demonstrated that extracts of this plant exhibited higher antimicrobial activity than *Trivalvaria macrophylla* and *Rennelia elliptica*. Significant inhibitory activity was observed against *Candida albicans*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Streptococcus sobrinus*, with a maximum activity index of 0.96 at a concentration of 1000 micrograms per well. Additional studies reported that ethanolic, acetone, and methanolic extracts from the leaves and stems were active against both Gram-positive bacteria such as *S. aureus*, *Micrococcus luteus*, *Enterococcus faecalis*, *Bacillus subtilis* and Gram-negative species such as *Proteus vulgaris*, *Escherichia coli*, and

Salmonella typhi [15]. Interestingly, extracts from the roots showed inconsistent antimicrobial activity [16, 17], which further emphasizes the importance of evaluating other plant parts, including flowers, for potential medicinal applications.

Hydrogel-based wound dressings must possess antimicrobial properties in order to protect wounds from pathogenic microorganisms and promote effective healing [18]. According to Chen, Fu [19], ideal wound dressings should prevent bacterial colonization, inhibit microbial growth within the wound area, and reduce inflammation. Common pathogens found in wound environments include *E. coli, S. aureus,* and *C. albicans*, which are frequently implicated in skin and soft tissue infections [20-22]. These concerns highlight the relevance of incorporating antimicrobial plant extracts into hydrogel formulations for improved wound care [23].

2. METHODOLOGY

2.1 Materials

The flower and leaf of *Eurycoma longifolia* Jack, Alginate, Polyethylene glycol 6000 (PEG), polyvinyl pyrrolidone (PVP) or Kollidon 25, 2-Phenoxyethanol, Propylene glycol (PG), Fetal Bovine Serum (FBS), Dulbecco's Modified Eagle Medium F12 (DMEM F12), ORL-48 cancer cells.

2.2 Method

2.2.1 Preparation of Plant Samples

Plant preparation was performed according to [24]. The fresh flowers and leaves of *E. longifolia* were washed thoroughly under running water to remove foreign material. The plant material was then air-dried at room temperature for seven days. After drying, the samples were ground into a fine powder using a blender to increase extraction efficiency.

2.2.2 Extraction of Flower and Leaf Samples

Methanolic extraction was performed using a maceration technique based on [25]. One hundred gram of powdered flower material and one hundred grams of powdered leaf material were placed into separate containers, and each was immersed in 1 L of methanol at a ratio of 1:10. The samples were kept at room temperature and stored in the dark to minimize photodegradation. Maceration was performed for 48 hours.

After the initial extraction period, the mixture was filtered, and the plant residue was resoaked in fresh methanol at the same ratio. This procedure was repeated three times to maximize extraction of secondary metabolites. The combined filtrates were passed through vacuum filtration using Whatman No. 1 filter paper.

The methanolic extracts were concentrated using a rotary evaporator set at 200 rpm and 40 °C to remove the solvent. To ensure complete drying, the concentrated extracts were placed in a fume hood for an additional 24 hours.

2.2.3 Hydrogel Formulation

Hydrogels were prepared using alginate, PVP, and PEG as the polymeric bases according to the formulations shown in Table 1. The total polymer content in each formulation was standardized at 3 % (w/w).

Each polymer was weighed accurately and dispersed in distilled water. The dispersion was allowed to hydrate for five to ten minutes so the polymers could swell and form a gel network. Propylene glycol (5 % (w/w)) was incorporated as a humectant and stabilizing agent, while 2-phenoxyethanol (1 % (w/w)) was added as a preservative. These components were mixed thoroughly.

Methanolic extracts of the flower or leaf samples were incorporated at a concentration of 3 % (w/w), corresponding to 0.6 g per formulation. The extracts were added to the hydrated polymer matrix and mixed uniformly. Distilled water was then added to achieve a final volume of 20 mL.

The mixture was continuously stirred using a magnetic stirrer at a temperature between 30 and 40 °C for fifteen minutes until a homogeneous hydrogel was formed.

Table 1 Hydrogels formulation from methanol extract of *E. longifolia* Jack flower and leaf

Formulation	F1	F2	F3	F4	F5	F6
Alginate (g)	0.6	0.45	0.45	0.6	0.45	0.45

Polyvinyl pyrrolidone (PVP) (g)	-	0.15	-	-	0.15	-
Polyethylene glycol (PEG) (g)	-	-	0.15	-	-	0.15
2-phenoxyethanol (1% w/w) (g)	0.2	0.2	0.2	0.2	0.2	0.2
Propylene glycol (5% w/w) (g)	1	1	1	1	1	1
Methanol extract of Pasak Bumi flower (3%) (g)	0.6	0.6	0.6	-	-	-
Methanol extract of Pasak Bumi leaf (3%) (g)	-	-	-	0.6	0.6	0.6
Distilled water	Up to 20 mL					

2.2.4 Characterization and Evaluation of Hydrogels

The hydrogel formulations were subjected to a comprehensive series of physicochemical, microbiological, and biological evaluations to assess their suitability as wound healing materials. The characterization procedures included physical analysis, chemical analysis, microbiological testing, and an in vitro wound healing scratch assay. All experiments were conducted in triplicate unless stated otherwise.

2.2.4.1 Physical Characterization of Hydrogels

a. Viscosity Analysis

The viscosity of each hydrogel formulation was determined to assess its rheological behavior and suitability for topical application. Approximately 20 g of each hydrogel sample were placed into a clean beaker. A rotational viscometer equipped with an appropriate spindle (selected based on sample consistency) was used to measure viscosity. The spindle was immersed in the hydrogel without introducing air bubbles. Measurements were recorded at a controlled temperature of 25 °C and at a fixed rotational speed until a stable reading was obtained. The viscosity values were expressed in centipoise. The analysis allowed comparison of flow characteristics among formulations containing different polymer ratios.

b. Spreadability Analysis

Spreadability was evaluated according to the general principles described by Garg, Aggarwal [26]. This test was performed to determine the ease with which the hydrogel spreads on the skin surface. A fixed quantity of hydrogel (approximately 1 g) was placed at the center of a glass plate. A second glass plate of known weight was gently placed on top to allow the hydrogel to spread. An additional weight was applied for one minute to ensure uniform spreading. The diameter of the spread hydrogel was measured in two perpendicular directions using a digital caliper. The average diameter was calculated and recorded as the spreadability value. Higher spreadability indicated easier application and better coverage on the skin.

c. Stability Assessment

Stability testing was conducted to observe potential physical or structural changes in the hydrogel over time. Samples were stored at room temperature and monitored periodically for changes in color, odor, homogeneity, phase separation, or microbial contamination. Hydrogel samples were inspected at predetermined intervals for up to four weeks. Any observable alteration in texture, consistency, or appearance was documented. Stable hydrogels were expected to maintain uniformity without precipitation, syneresis, or changes in odor.

2.2.4.2 Chemical Characterization of Hydrogels

a. pH Analysis

The pH of each hydrogel formulation was measured to determine its compatibility with the natural pH range of human skin. The analysis followed Chemists [27] methods. A sample of hydrogel (approximately 5 g) was dispersed in 45 mL of distilled water in a beaker. The mixture was stirred gently to ensure uniform dispersion. A calibrated digital pH meter with a glass electrode was inserted into the sample and allowed to stabilize before recording the reading. The pH measurements were performed in triplicate for accuracy. Formulations intended for dermal use must typically exhibit a pH between 4.5 and 7.0 to avoid skin irritation.

b. Water Content Analysis

Water content was determined using Chemists [27] procedures to evaluate the hydration capacity and moisture retention properties of the hydrogel. Approximately 2 g of hydrogel were weighed into a moisture analysis dish and placed in a drying oven set at a controlled temperature until a constant weight was achieved. The weight loss during the drying process represented the water content in the sample. The percentage of water content was calculated using the formula:

$$Water\ content\ (\%) = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100$$

Hydrogels with appropriate water content maintain moisture at the wound site and support the healing process.

2.2.4.3 Antibacterial Activity Testing

Antibacterial activity of the hydrogel formulations was assessed following the procedure described by [28]. Selected bacterial strains commonly associated with wound infections, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, were cultured on nutrient agar plates and incubated at 37 °C for 24 hours.

Hydrogel samples were prepared in uniform discs using sterile molds and placed onto freshly inoculated agar plates. Plates were incubated at 37 °C for another 24 hours. After incubation, the zones of inhibition surrounding each hydrogel disc were measured using a digital caliper. The diameter of the inhibition zone represented the antimicrobial potency of the formulation. Control plates without hydrogel treatment were included to validate bacterial growth. Results were recorded as mean inhibition zone diameters.

2.2.5 In Vitro Wound Healing Analysis Using Scratch Assay

The wound healing potential of the hydrogel extracts was examined using an in vitro scratch assay adapted from [29]. The ORL-48 oral cancer cell line was used to assess cell migration in the presence of flower and leaf extracts.

2.2.5.1 Cell Preparation

Cells were cultured in complete DMEM F12 medium supplemented with 20 % fetal bovine serum (FBS). The cells were seeded into six-well plates and incubated for 48 hours at 37 °C in a humidified incubator containing 5 % carbon dioxide until they reached confluence.

2.2.5.2 Scratch Formation

A sterile 200 µL pipette tip was used to create a straight scratch across the monolayer to simulate a wound gap. Detached cells were removed by washing the wells with phosphate-buffered saline.

Four treatment groups were prepared as below:

- Methanolic flower extract at 200 µg/mL in DMEM F12 medium
- Methanolic leaf extract at 200 µg/mL in DMEM F12 medium
- Negative control containing DMEM F12 medium without extract
- Positive control containing complete medium with 20 % FBS

Each treatment was applied to triplicate wells. The plates were incubated at 37 °C in 5 % carbon dioxide for 48 hours.

The scratch area was observed at 24 hours and 48 hours under a microscope at 100 times magnification. Images were captured using a digital imaging system. The wound closure percentage was calculated by quantifying the reduction in scratch width using NIH ImageJ software. Increased cell migration into the scratch area indicated enhanced wound healing activity of the extract.

2.2.6 Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA) with Minitab 17 (Minitab, LLC, Pennsylvania, US). Significant differences among formulations were evaluated using Tukey pairwise comparison at a 95 % confidence level ($\alpha = 0.05$). The optimal hydrogel formulation was identified using a Multiple Attribute Decision Method.

3. RESULTS AND DISCUSSION

3.1 Methanolic Extracts Yield of E. longifolia Flowers and Leaves

The flowers and leaves of *E. longifolia* exhibited markedly different extraction yields following methanolic maceration. The flower

extract produced a yield of 55.7 %, while the leaf extract produced a significantly lower yield of 9.72 %. This large disparity indicates pronounced differences in the chemical composition of the two plant parts.

Flower tissues typically accumulate pigments, glycosylated flavonoids, sugars, hydrophilic phenolics, and other polar components that dissolve efficiently in methanol. These constituents likely contributed to the higher extractive yield observed. In contrast, the leaves contain comparatively fewer highly polar compounds and more structural fibers, which decreased the proportion of methanol-extractable material. The high yield of the flower extract therefore supports the hypothesis that the flowers of *E. longifolia* are a rich but underexplored source of phytochemicals that may play important physiological roles in the plant itself and offer potential bioactivity for human health applications.

3.2 Phytochemical Analysis and Antioxidant Properties of Flowers and Leaves Extract

3.2.1 Total Polyphenol and Total Flavonoid Content

The methanolic flowers extract contained 5.78 mg GAE/g of total phenolics and 0.79 mg QE/g of total flavonoids. Meanwhile, for the leaves extract, the contents of total phenol and total flavonoid are 31.2 ± 1.8 mg GAE/g and 4.3 ± 0.4 mg QE/g, respectively (Table 2). Although total flavonoid content was relatively low, the detectable levels confirm the presence of flavonoid-derived antioxidants.

Table 2 Summary of phytochemical analysis of *E. longifolia* flowers and leaves extract.

Compound	Flower extract	Leaves extract
Total phenol content (mg GAE/g)	5.78 ± 0.053	31.2 ± 1.8
Total flavonoid content (mg QE/g)	0.79 ± 0.017	4.3 ± 0.4
IC_{50} value (ppm)	11.604 ± 1.1	4.2 ± 0.5

The phenolic concentration suggests that the flower contains moderate levels of compounds capable of donating electrons or hydrogen atoms to neutralize oxidants. Polyphenols are well documented for their roles in radical scavenging and metal chelation and are widely regarded as key contributors to antioxidant activity in plants.

The lower flavonoid content suggests that the methanolic flower extract may be dominated by other phenolic classes or lipophilic antioxidant constituents such as triterpenoids or steroids. Previous phytochemical reports on other plant parts support the presence of such compounds in *E. longifolia*, which further aligns with the current results.

The purple-red coloration of the flowers observed during sample preparation suggests the presence of anthocyanins, which are water soluble flavonoids responsible for red to purple pigmentation. Anthocyanins are known for their strong antioxidant activity and high solubility in methanol. This aligns with the observed color and the measurable phenolic content.

3.2.2 Antioxidant Capacity by DPPH Assay

The antioxidant capacity of the flowers and leaves extracts were evaluated using the DPPH radical scavenging method. The extract exhibited an IC_{50} value of 11.604 ± 1.1 ppm and 4.2 ± 0.5 for the flowers and leaves extracts, respectively (Table 2), which falls within the classification of strong antioxidant activity, where IC_{50} values below 50 ppm indicate high potency. The capacity to reduce the purple DPPH radical to its yellow hydrazine form demonstrates the ability of the extract to donate electrons or hydrogen atoms.

The strong antioxidant effect is significant because antioxidants play essential roles in mitigating oxidative stress, which contributes to cellular damage, chronic inflammation, and carcinogenesis. The result supports the proposition that *E. longifolia* flower and leave extracts may possess biological activities relevant to dermatological applications, including wound healing, where oxidative damage can delay tissue regeneration.

3.3 Physicochemical Properties of Hydrogel Formulations

The extracts were incorporated into hydrogels prepared with different polymer compositions to evaluate their physical suitability as wound dressing materials. Across all formulations,

hydrogels displayed homogeneous textures and characteristic colors reflective of the incorporated extracts. The physical characteristics observed provide important preliminary insights into the compatibility of the extract with common hydrogel polymers.

3.3.1 Viscosity Analysis

Viscosity plays a central role in determining the clinical usability of hydrogels. High viscosity typically provides structural firmness, while low viscosity improves spreadability and facilitates uniform application on irregular wound surfaces.

The results from rheological testing revealed clear effects of polymer composition on viscosity. Hydrogels containing alginate as the sole polymer exhibited the highest viscosity values. This is consistent with the known crosslinking behavior of alginate molecules in aqueous systems, where intermolecular associations create a dense three-dimensional network that resists flow.

In contrast, formulations containing PVP or PEG demonstrated markedly reduced viscosity (Figure 2). Both polymers have lower intrinsic gel-forming capacity, and their incorporation into the matrix likely diluted the networking density created by alginate, producing softer and more flexible gels. PEG is highly hydrophilic and decreases internal friction within the gel, while PVP contributes to solubilization and reduces intermolecular bonding intensity.

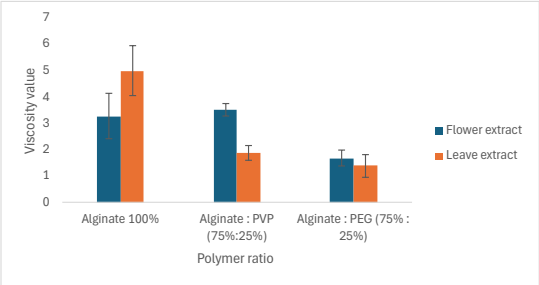

Figure 2 Viscosity analysis of hydrogels.

The viscosity values ranged from 1.394 Pa.s to 4.971 Pa.s. The lowest viscosity was observed in the alginate:PEG (75:25) hydrogel with leaf extract (F6). The highest viscosity was the alginate-only hydrogel with leaf extract (F4). These results show that the leaf extract did not interfere with polymer gelation significantly except in the case of alginate:PVP hydrogels, where extract interactions may have caused minor deviations.

Lower viscosity formulations are desirable for wound healing because they spread more effectively and provide better contact with the wound surface. These findings therefore support the potential clinical utility of mixed-polymer hydrogels, especially those containing PEG.

3.3.2 Spreadability Analysis

Spreadability is a crucial performance parameter in topical formulations because it determines ease of application and uniformity of therapeutic delivery. There was a consistent inverse relationship between viscosity and spreadability across all hydrogel types (Figure 3).

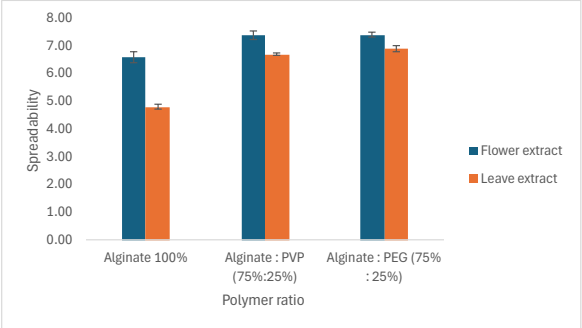

Figure 3 Spreadability of hydrogels.

Hydrogels with lower viscosity displayed higher spreadability values. The alginate:PEG (75:25) hydrogel with flower extract achieved a spreadability value of 7.40 g·cm per second, which was the highest among all formulations. Conversely, alginate-only hydrogels, which exhibited the highest viscosity, had the lowest spreadability.

The spreadability values obtained in this study fall within ranges reported in previous gel formulation studies, indicating that the hydrogels produced have acceptable physical characteristics for topical use. High spreadability ensures uniform coverage of the wound surface, promotes optimal drug release, and improves patient compliance.

3.3.3 Stability Analysis

Stability testing over 21 days demonstrated that all hydrogels retained their color throughout the study period (Figure 4). This suggests that the pigments present in the extracts are stable within the hydrogel matrix under ambient conditions.

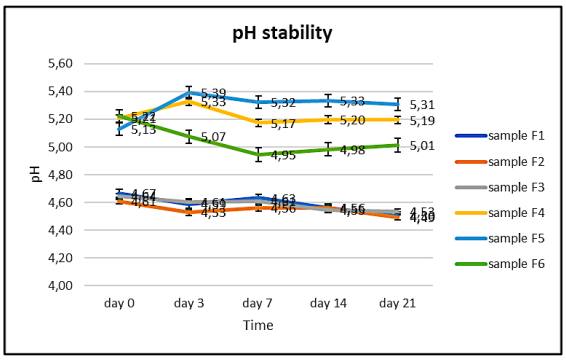

Figure 4 pH stability of hydrogels.

Homogeneity remained consistent for all formulations until day 7. Minor changes were observed in formulations F2, F5, and F6 by day 14 and day 21, where white particulates or slight drying were noted. These changes were reversible or minimal, indicating that the formulations remained structurally stable and that no significant phase separation or degradation occurred.

The hydrogels composed primarily of alginate (F1, F3, F4) were the most stable throughout the entire 21 days. This suggests that alginate provides stronger polymeric support and enhanced resistance to dehydration compared to the PVP or PEG containing systems.

The stability results overall demonstrate that the hydrogels maintain acceptable physicochemical integrity for at least three weeks, which is sufficient for most topical storage and usage periods.

3.4 Chemical Characteristics of Hydrogels

3.4.1 Water Content

Water content ranged between 6.68 and 6.87 % for all formulations (Figure 5). There were no significant differences between hydrogels containing flower or leaf extracts. Alginate-only formulations exhibited the lowest water content due to the natural tendency of alginate gels to undergo syneresis, which results in expulsion of water from the polymer matrix.

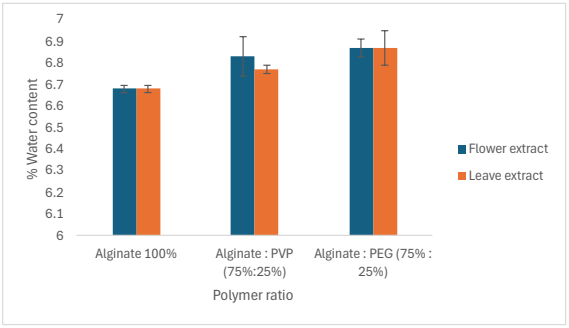

Figure 5 Water content of hydrogel.

Hydrogels containing PEG presented the highest water content values. PEG contains multiple hydrophilic ethylene oxide units that retain water, reducing evaporation and enhancing moisture retention. Moisture retention is critical for wound healing because a moist environment promotes fibroblast proliferation, enzymatic activity, and epithelial regeneration.

Given the narrow water content range observed, all formulations fall within acceptable limits for hydrogel wound dressings.

4.4.2 pH Analysis

The pH values of all hydrogel formulations ranged from 4.33 to 5.08 (Figure 6). This pH range is ideal for topical application because human skin generally maintains an acidic mantle between pH 4.5 and 6.5.

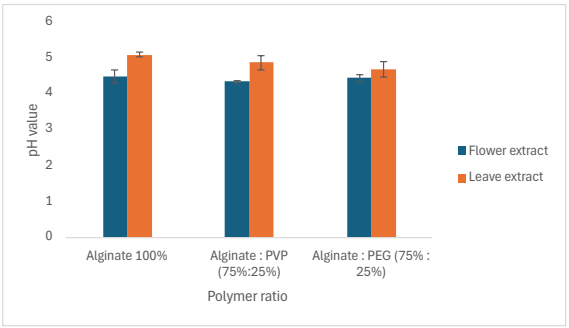

Figure 6 pH value of hydrogels.

Slightly acidic hydrogels are advantageous for wound healing because they inhibit microbial colonization, enhance the activity of certain enzymes involved in tissue repair, and improve dermal penetration of bioactive compounds. The differences observed among formulations were minor and were likely due to inherent chemical differences between the flower and leaf extracts.

All formulations exhibited pH values consistent with safe and effective application to the skin.

4.5 Antimicrobial Characteristics

The antimicrobial activity of the hydrogels was assessed against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. These organisms represent common wound contaminants and are important indicators of antimicrobial efficacy.

4.5.1 Antibacterial Activity Against E. coli

Most hydrogels demonstrated high antimicrobial activity against *E. coli*, with kill percentages ranging from 74.30 % to 100 % (Table 3). Formulations F3 and F5 showed slightly lower activity, potentially due to interactions between the extract components and polymer matrix that may have slowed release of antimicrobial constituents.

Table 3 The kills percentage of each hydrogel against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

Sample	Hydrogel formulation	*E. coli* Mean ± SD	*S. aureus* Mean ± SD	*C. albicans* Mean ± SD
Flower extract	F1	100.00 ± 0.000	100.00 ± 0.000	100 ± 0.000
	F2	100.00 ± 0.000	100.00 ± 0.000	100 ± 0.000
	F3	93.33 ± 11.547	100.00 ± 0.000	100 ± 0.000
	F4	100.00 ± 0.000	96.67 ± 5.774	100 ± 0.000
Leave extract	F5	74.30 ± 28.974	100.00 ± 0.000	100 ± 0.000
	F6	91.67 ± 14.434	100.00 ± 0.000	100 ± 0.000
Negative control (-)	KN1	95.23 ± 8.256	100.00 ± 0.000	100 ± 0.000
	KN2	100.00 ± 0.000	100.00 ± 0.000	100 ± 0.000
	KN3	100.00 ± 0.000	100.00 ± 0.000	100 ± 0.000
Positive control (-)	KP	0.00 ± 0.000	0.00 ± 0.000	0 ± 0.000

Gram negative bacteria such as *E. coli* possess an outer membrane with high lipid content, which can impede the penetration of many antibacterial agents. The strong antibacterial activity observed, even in this more resistant organism, suggests effective diffusion of antimicrobial compounds from the hydrogel matrix [30].

4.5.2 Antibacterial Activity Against S. aureus

All formulations exhibited almost complete inhibition of *S. aureus* (Table 3). The consistent 100 percent kill rate indicates that the hydrogels are highly effective against Gram positive bacteria. Gram positive cell walls contain thick peptidoglycan layers that are more susceptible to disruption by phytochemicals, which supports the results observed.

These findings closely match earlier studies reporting strong antibacterial activity of *E. longifolia* extracts against *S. aureus [31]*.

4.5.3 Antifungal Activity Against C. albicans

All formulations, including negative controls, showed 100% kill against *C. albicans* (Table 3). This uniform activity is attributed to 2-phenoxyethanol, the preservative incorporated in all hydrogels. The compound disrupts fungal respiration and enzymatic systems, leading to cell death.

The strong antifungal activity confirms that the hydrogels are capable of suppressing growth of one of the most frequent fungal pathogens associated with wound infections [32, 33].

4.6 Selection of Best Hydrogel Formulation

A Zeleny multiple-attribute decision analysis was applied using viscosity, spreadability, water content, pH, and antimicrobial activity as selection criteria. The analysis identified F6, the alginate:PEG (75:25) hydrogel containing leaf extract, as the optimal formulation.

F6 demonstrated the lowest viscosity, high spreadability, appropriate water content, optimal pH, and strong antimicrobial activity. These combined attributes indicate that F6 possesses the most balanced physicochemical and biological properties for potential wound healing applications.

4.7 In Vitro Wound Healing Scratch Assay

The wound healing potential of flower and leaf extracts was evaluated using ORL-48 cells, a human oral carcinoma cell line.

4.7.1 Performance of Controls

The positive control (complete medium) showed rapid and complete wound closure within 24 hours, confirming normal proliferative and migratory behavior of the cell line (Figure 4.8). The negative control (serum-free medium) exhibited reduced migration and incomplete wound closure, as expected under nutrient-deprivation conditions.

4.7.2 Effects of Extracts on Cell Migration and Viability

Both flower (EB) and leaf (ED) extracts inhibited wound closure and induced pronounced cytotoxicity. Instead of reducing the scratch gap, the treated wells showed increasing wound area over 48 hours, accompanied by floating white cells that are indicative of cell death. Quantitative wound contraction values for EB and ED were negative (-30.76 % and -47.48 %, respectively), confirming that the extracts inhibited migration and reduced the number of viable cells in the monolayer (Figure 7). The stronger inhibitory effect of the leaf extract suggests a higher concentration or potency of cytotoxic metabolites [34]. These results are consistent with reports that *E. longifolia* contains quassinoids and related compounds that possess cytotoxic properties [35].

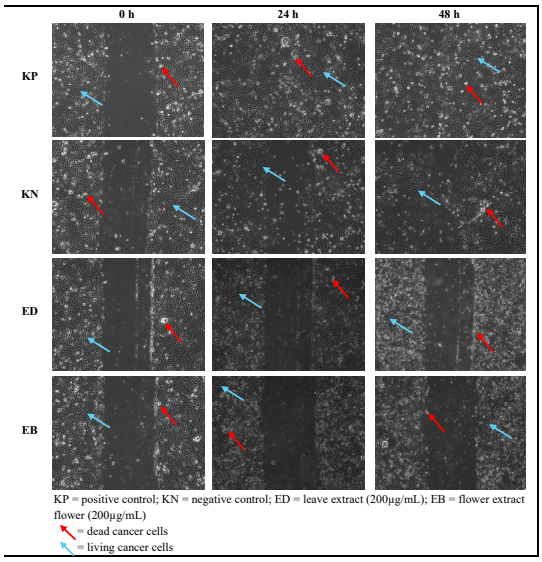

Figure 7 Migration and healing effect with ORL-48 scratch assay method.

4.7.3 Implications for Antimetastatic Activity

The suppression of ORL-48 cell migration and proliferation indicates that the extracts interfere with cellular processes critical for metastasis, such as cytoskeletal remodeling and energy metabolism. The rapid induction of cell death in treated wells suggests potential application of the extracts in cancer research [36], although further studies are needed to evaluate selectivity toward tumor cells compared to normal cells [37].

5. CONCLUSION

This study demonstrated that the flowers and leaves of *E. longifolia* possess distinct phytochemical characteristics and exhibit promising biological properties when incorporated into hydrogel formulations for potential wound care applications. The methanolic flower extract produced a substantially higher yield than the leaf extract, indicating a greater abundance of methanol soluble constituents [38]. The flower extract also showed measurable phenolic and flavonoid contents and demonstrated strong antioxidant activity with an IC_{50} value of 11.604 ppm. Figure 8 summarized the formulation and biological activity of this extract.

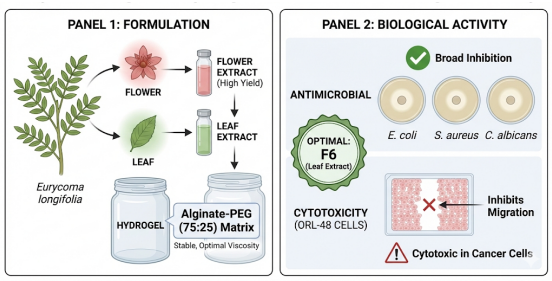

Figure 8 The *E. longifolia* hydrogel formulation and its biological activity.

Hydrogels formulated with alginate, PVP, and PEG displayed acceptable physical, chemical, and microbiological properties. Polymer composition significantly influenced viscosity and spreadability, with alginate:PEG (75:25) formulations exhibiting the most favorable rheological profile for topical application. All hydrogels maintained satisfactory stability over 21 days and presented water content and pH values within acceptable ranges for dermal use. Antimicrobial evaluation showed that the hydrogels were effective against *E. coli*, *S. aureus* and *C. albicans*, with strong inhibitory activity observed in most formulations. The antimicrobial effect observed in all formulations, including controls, was partly attributed to 2 phenoxyethanol contained in the base formulation.

Using a multi attribute decision analysis, the alginate:PEG (75:25) hydrogel containing leaf extract (F6) was identified as the optimal formulation due to its balanced physicochemical properties and strong antimicrobial performance [39]. The in vitro scratch assay indicated that both flower and leaf extracts inhibited the proliferation and migration of ORL-48 cancer cells, which suggests the presence of cytotoxic constituents in these extracts. Although this activity is not suitable for promoting wound closure in cancerous cell models, it provides important insight into the broader bioactivity profile of *E. longifolia* extracts.

Overall, the findings support the feasibility of incorporating *E. longifolia* flower and leaf extracts into hydrogel matrices and provide a foundation for further investigation using non-cancerous dermal or fibroblast cell models to determine their true wound healing potential [40]. Additional studies involving cytocompatibility, controlled release behavior, and *in vivo* wound healing assessment are necessary before clinical or commercial application can be considered [41].

Abbreviation

Eurycoma longifolia (*E. longifolia*); Staphylococcus aureus (*S. aureus*); *Escherichia coli* (*E. coli*); *Candida* albicans (C. albicans); Streptococcus mutans (S. mutans); Streptococcus sobrinus (*S. sobrinus*); *Micrococcus luteus* (*M. luteus*); *Enterococcus faecalis* (*E. faecalis*); *Bacillus subtilis* (*B. subtilis*); *Proteus vulgaris* (*P. vulgaris*); *Salmonella typhi* (*S. typhi*); Polyvinyl pyrrolidone (PVP); Polyethylene glycol (PEG); Propylene glycol (PG); Fetal bovine serum (FBS); Dulbecco's Modified Eagle Medium F12 (DMEM F12); Gallic acid equivalent (GAE); Quercetin equivalent (QE); Half-maximal inhibitory concentration (IC50); 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Author Contributions

Conceptualization, methodology, and study design: A.S.M. Sabere. Experimental work, data acquisition, and analysis: A.S.M. Sabere, F. Malau, M.H. Arzmi. Data interpretation, figure preparation, and manuscript drafting: A.S.M. Sabere, F. Malau. Critical review, data interpretation, and final editing: M.Z. Sadikan. All authors have read and approved the final manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

Data will be made available upon request to the corresponding authors.

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Conflicts of Interest

The authors declare no conflicts of interest. All authors have contributed substantially to the conception, design, drafting of the article, and in final approval of the version of the manuscript to be submitted. All authors have jointly decided to designate A.S.M. Sabere to be responsible for decision-making regarding the presence of authors and the order of their presence in the manuscript. A.S.M. Sabere has also been selected by all authors to be responsible for all future communication with the journal regarding this manuscript. Additionally, M.Z. Sadikan have been appointed as co-corresponding author.

References

1. Mutiah R, Handayani R. Potential and mechanisms of indigenous Indonesian medicinal plants in treating sexual dysfunction: A systematic review and pharmacological network overview. Heliyon. 2025;11(3).
2. Rehman SU, Choe K, Yoo HH. Review on a traditional herbal medicine, Eurycoma longifolia Jack (Tongkat Ali): its traditional uses, chemistry, evidence-based pharmacology and toxicology. Molecules. 2016;21(3):331.
3. Yance DR. Adaptogens in medical herbalism: elite herbs and natural compounds for mastering stress, aging, and chronic disease: Simon and Schuster; 2013.
4. Khanijo T, Jiraungkoorskul W. Review ergogenic effect of long jack, Eurycoma Longifolia. Pharmacognosy Reviews. 2016;10(20):139.
5. Ayob Z, Wagiran A, Abd Samad A. Potential of tissue cultured medicinal plants in Malaysia. Jurnal Teknologi (Sciences & Engineering). 2013;62(1).
6. Parikrama R, Esyanti RR. Effect of UV elicitation on callus growth, alkaloid and terpenoid contents in Eurycoma longifolia Jack. Int J Adv Chem Eng Biol Sci. 2014;1(1):12–5.
7. Astuti KEW, Handajani SR. Efektifitas Anti Inflamasi Formulasi Kunyit (Curcuma Longa), Daun Binahong (Anredera Cordifolia) Dan Daun Sambiloto (Andrographis Paniculata) Terhadap Luka Sayat Pada Kelinci. Interest: Jurnal Ilmu Kesehatan. 2018;7(2):211–21.
8. Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. Journal of international medical research. 2009;37(5):1528–42.
9. Alberts A, Moldoveanu E-T, Niculescu A-G, Grumezescu AM. Hydrogels for wound dressings: applications in burn treatment and chronic wound care. Journal of Composites Science. 2025;9(3):133.
10. Erizal E, Chosdu R. Pengekangan Obat Dalam Matriks Hidrogel PVA-ko-NIPAAm Hasil iradiasi. 1997.
11. Ostrovidova G, Makeev A, Shamtsian M. Polyfunctional film coatings for medical use. Materials Science and Engineering: C. 2003;23(4):545–50.
12. Kumakura M, Kaetsu I. Immobilization of cells by radiation copolymerization of hydrophilic and hydrophobic monomers. ACTA CHIMICA HUNGARICA-MODELS IN CHEMISTRY. 1984;116(4):345–51.
13. Utomo BSB, Fransiska D, Darmawan M. Formulasi hidrogel dari polivinil pirolidon dan k/i-karaginan untuk bahan pembalut luka. Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan. 2016;11(1):55–66.
14. Kuspradini H, Silau S, Supartini S, Rosamah E, ICTROPS. Comparative antimicrobial studies on plant species known as' Pasak Bumi': Eurycoma longifolia Jack., Rennelia elliptica Korth. and Trivalvaria macrophylla miq. F1000Research. 2019;8:301.
15. Farouk A, Benafri A. Antibacterial activity of Eurycoma longifolia Jack. Saudi Med J. 2007;28(9):1422–4.
16. bin Ramzi MI, bin Kosnin MH, Faisal GG, Arzmi MH, Kusumawardani A, Sabere A, et al. The effect of Eurycoma longifolia Jack (Tongkat Ali) root extract on salivary S. mutans, Lactobacillus and Candida albicans isolated from high-risk caries adult patients. Pharmacognosy Journal. 2021;13(3).
17. Latip MQA, Noor MHM, Ahmad H, Hassim HA, Salleh A, Bejo MH, et al. A systematic review on antimicrobial and antiparasitic activity of Eurycoma Longifolia Jack (Tongkat Ali). BioMed Research International. 2022;2022(1):4999797.
18. Alberts A, Tudorache D-I, Niculescu A-G, Grumezescu AM. Advancements in wound dressing materials: Highlighting recent progress in hydrogels, foams, and antimicrobial dressings. Gels. 2025;11(2):123.
19. Chen S-L, Fu R-H, Liao S-F, Liu S-P, Lin S-Z, Wang Y-C. A PEG-based hydrogel for effective wound care management. Cell transplantation. 2018;27(2):275–84.
20. Zhang S, Zhu Y, Zou J, Zhang M, Li X, Wang Y, et al. Screening of an antibacterial peptide with high antibacterial activity against superbugs

and its application in the treatment of skin wound infections. Chemical Engineering Journal. 2025:167748.
21. Al-Shami AS, Alzomor M. Antimicrobial Activity of the Latex of Jatropha Curcas Against Cutaneous Wound and Burns Infection. Infection and Drug Resistance. 2025:3591–603.
22. Öztürk BY, Çiğdem U, Gürsu BY, Dağ İ. Cyanobacterium Chroococcus sp. Mediated Silver Nanoparticles to Fight Candida albicans and Staphylococcus aureus Biofilms: Effect of Dual Species Conditions. Applied Biochemistry and Biotechnology. 2025:1–33.
23. Usama S, Riaz M, Ali B, Khan DS, Ahmad S, Ahmad Z. Hydrogel-Plant Extract Composites in Wound Healing. Phytopharmacology Research Journal. 2024;3(2):26–9.
24. Aggarwal H, Ghosh J, Rao A, Chhokar V. Evaluation of root and leaf extracts of Glycrriza glabra for antimicrobial activity. J Med Biol Eng. 2015;4(1).
25. Zhang Q-W, Lin L-G, Ye W-C. Techniques for extraction and isolation of natural products: A comprehensive review. Chinese medicine. 2018;13(1):20.
26. Garg A, Aggarwal D, Garg S, Singla AK. Spreading of semisolid formulations: an update. Pharmaceutical Technology North America. 2002;26(9):84–.
27. Chemists AoOA. Official methods of analysis of the Association of Official Analytical Chemists: The Association; 2000.
28. Chen H, Cheng J, Ran L, Yu K, Lu B, Lan G, et al. An injectable self-healing hydrogel with adhesive and antibacterial properties effectively promotes wound healing. Carbohydrate polymers. 2018;201:522–31.
29. Chen Y. Scratch wound healing assay. Bio-protocol. 2012;2(5):e100–e.
30. Tang Y, Xu H, Wang X, Dong S, Guo L, Zhang S, et al. Advances in preparation and application of antibacterial hydrogels. Journal of Nanobiotechnology. 2023;21(1):300.
31. Liu Y-X, An X-L, Xu Y-N, Hao Y-J, Piao X-C, Jin M-Y, et al. Antibacterial and antibiofilm properties of dichloromethane fraction of extracts from adventitious roots of Eurycoma longifolia against Staphylococcus aureus. LWT. 2022;162:113438.
32. Zhou D, Li X, Zhu Y, Hu Y, Zhang S, Tong Z, et al. Fungal-derived chitosan-based hydrogels with antimicrobial properties for infectious wound healing. Carbohydrate Polymers. 2025:123917.
33. Concha V, Díaz-Dosque M, Duarte LF, Jara JA, Molina-Berríos A. Characterization of Hydrogel Beads for the Gradual Release of Origanum vulgare L. Essential Oil and Evaluation of Their Antifungal Activity Against Candida albicans. Microorganisms. 2025;13(9):2065.
34. Bramki A, Barboucha G, Benslama O, Seglab F, Makhlouf FZ, Nessah S, et al. Therapeutic Insights into Rubus ulmifolius Schott Leaf Extract: In Vitro Antifungal, Enzyme Inhibition, and Anticancer Activities Integrated with Network Pharmacology and Molecular Docking Analyses of Colorectal and Ovarian Cancer. Pharmaceuticals. 2025;18(10):1563.
35. Mansyah B. Diversity of Secondary Metabolites of Eurycoma Longifolia and Its Prospects as Anticancer Agents Based on Sumatran Bioresources: A review. Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam. 2025;22(1):80–94.
36. Albahri G, Badran A, Abdel Baki Z, Alame M, Hijazi A, Daou A, et al. Potential anti-tumorigenic properties of diverse medicinal plants against the majority of common types of cancer. Pharmaceuticals. 2024;17(5):574.
37. Hołota M, Posmyk MM. Nature-Inspired Strategies in Cancer Management: The Potential of Plant Extracts in Modulating Tumour Biology. International Journal of Molecular Sciences. 2025;26(14):6894.
38. Tourabi M, Faiz K, Ezzouggari R, Louasté B, Merzouki M, Dauelbait M, et al. Optimization of extraction process and solvent polarities to enhance the recovery of phytochemical compounds, nutritional content, and biofunctional properties of Mentha longifolia L. extracts. Bioresources and Bioprocessing. 2025;12(1):24.
39. Hamid LL, Zakir RS, Abdel Rahman NM, Idrahim RH, Abdel Rahman SM. Construction and Characterization of Polyethylene Glycol/Sodium Alginate Hydrogel Loaded With Zirconia Nanoparticles: Potential Antibacterial and Antibiofilm Agent to Inhibit Dental Caries In Vitro and In Vivo. Microscopy Research and Technique. 2025.
40. Al-Bayati MRY, Faisal GG, Abd Fuaat A, Affandi KA, Abidin MAZ. The effect of eurycoma longifolia jack tongkat ali hydrogel on wound contraction and re-epithelialization in in vivo

excisional wound model. Open Access Macedonian Journal of Medical Sciences. 2022;10(A):634–43.

41. Yaseen MR, Faisal GG, Abd Fuaat A, Affandi KA, Alallam B, Nasir MHM. Preparation of Euyrycoma longifolia Jack (EL) Tongkat Ali (Ta) root extract hydrogel for wound application. Pharmacognosy Journal. 2021;13(6).