https://doi.org/10.48047/AFJBS.6.13.2024.7059-7072

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Curcumin and Moringa-Infused Liposomal Hydrogel: A Novel Approach to Wound Healing

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Volume 6, Issue 13, Aug 2024

Received: 15 June 2024

Accepted: 25 July 2024

Published: 15 Aug 2024

doi: 10.48047/AFJBS.6.13.2024.7059-7072

Abstract:

This study investigates the formulation and evaluation of a liposomal hydrogel composed of curcumin and Moringa oleifera leaf extract for wound healing. Curcumin, a compound known for its anti-inflammatory and antioxidant properties, and quercetin, an active constituent of moringa leaves with similar properties, were encapsulated in liposomes using the thin film hydration method. The liposomes were incorporated into a carboxymethyl cellulose (CMC) hydrogel matrix. The encapsulation efficiency, particle size, zeta potential, surface morphology, swelling behavior, and release profile of the bioactive compounds were evaluated. The results indicated high encapsulation efficiencies of 88% for curcumin and 93.2% for moringa extract, with sustained release over 24 hours. The formulation showed promising potential for enhancing wound healing through prolonged bioavailability of the active agents and maintaining a moist wound environment.

Keywords: Curcumin, Moringa oleifera, liposomal hydrogel, wound healing, encapsulation efficiency

1.Introduction:

The skin, as the largest organ of the human body, serves multiple vital functions, including protection against pathogens, regulation of body temperature, and prevention of water loss(Abdo, Sopko and Milner 2020). It is composed of three primary layers: the epidermis, dermis, and hypodermis. The epidermis, the outermost layer, provides a barrier to infection and regulates water loss. The dermis, located beneath the epidermis, contains connective tissue, hair follicles, and sweat glands, while the hypodermis consists primarily of fat and connective tissue, providing insulation and cushioning(McKnight, Shah and Hargest 2022). A wound is defined as a disruption of the normal anatomical structure and function of the skin. Wound healing is a complex, dynamic process that can be categorized into four overlapping stages: hemostasis, inflammation, proliferation, and remodeling(Gupta 2021). Hemostasis occurs immediately after injury, where blood vessels constrict, and clot formation begins to prevent blood loss. The inflammatory phase follows, characterized by the recruitment of immune cells to the wound site to prevent infection. The proliferative phase involves the formation of new tissue, angiogenesis, and re-epithelialization. Finally, during the remodeling phase, the newly formed tissue undergoes maturation and reorganization to regain tensile strength and function(Flynn, Mahmoud et al. 2023). Curcumin, a polyphenolic compound derived from the rhizomes of Curcuma longa (turmeric), has been extensively studied for its therapeutic properties. It exhibits potent anti-inflammatory, antioxidant, and antimicrobial activities, making it a promising agent for wound healing(Fuloria, Mehta et al. 2022). However, curcumin's clinical application is limited by its poor solubility in water and low bioavailability, necessitating advanced delivery systems to enhance its therapeutic efficacy(Jyotirmayee and Mahalik 2022). Moringa oleifera, commonly known as the drumstick tree, is renowned for its nutritional and medicinal benefits. Moringa leaves are rich in vitamins, minerals, and bioactive compounds, including quercetin(Kashyap, Kumar et al. 2022). Quercetin is a flavonoid with significant antioxidant and anti-inflammatory properties. It has been shown to promote wound healing by modulating various cellular processes such as cell proliferation and migration(Carvalho, Araújo-Filho et al. 2021). Isolating quercetin from moringa leaves involves aqueous maceration followed by purification techniques like column chromatography(Devaraj 2023). Liposomes are spherical vesicles composed of phospholipid bilayers, capable of encapsulating both hydrophilic and hydrophobic substances(Farooque, Wasi and Mughees 2021).

They are widely used as drug delivery systems due to their biocompatibility, ability to encapsulate a wide range of therapeutic agents, and capacity to enhance the bioavailability of poorly soluble drugs(Venkatesh 2022). The thin film hydration method is a common technique for liposome preparation, involving the formation of a lipid film followed by hydration with an aqueous solution, leading to the formation of liposomes(Dua, Rana and Bhandari 2012). Hydrogels are three-dimensional, hydrophilic polymer networks that can absorb and retain substantial amounts of water. They are used in wound care due to their ability to maintain a moist environment, which is conducive to healing(Norahan, Pedroza-González et al. 2023). Hydrogels can be formulated from various polymers, including natural and synthetic ones. In this study, carboxymethyl cellulose (CMC) and citric acid were used to create a hydrogel matrix(Mali, Dhawale et al. 2018). The incorporation of liposomes into hydrogels combines the advantages of both delivery systems, providing sustained release of encapsulated drugs and maintaining a moist wound environment(Thirumaleshwar, K Kulkarni and V Gowda 2012). This study aims to formulate and evaluate a liposomal hydrogel containing curcumin and moringa leaf extract-derived quercetin for wound healing applications. The encapsulation of these bioactive compounds in liposomes is intended to enhance their stability and bioavailability, while the hydrogel matrix aims to provide a conducive environment for wound healing. The study involves the preparation of liposomes using the thin film hydration method, incorporation of liposomes into a hydrogel matrix, and comprehensive evaluation of the formulation's physicochemical properties and wound healing efficacy.

2. Materials and Methods

2.1. Materials:

Materials:

Curcumin was procured from [supplier name]. Moringa leaves were sourced from [supplier name] and authenticated by [authority name]. Phosphatidylcholine, cholesterol, carboxymethyl cellulose (CMC), citric acid, phosphate buffer saline (PBS), n-butanol, acetic acid, and chloroform were purchased from [supplier names]. All chemicals and reagents were of analytical grade and used without further purification.

Preparation of Curcumin and Moringa Leaf Extract Liposomes:

Curcumin and Moringa leaf extract liposomes were prepared using the thin-film hydration method. Briefly, 200 mg of phosphatidylcholine and 100 mg of cholesterol were dissolved in 10 mL of chloroform in a round-bottom flask. Curcumin (50 mg) and Moringa leaf extract (100 mg) were added to the lipid solution. The organic solvents were evaporated under reduced pressure using a rotary evaporator (Heidolph, Germany) at 40°C, forming a thin lipid film on the flask walls. The film was hydrated with 10 mL of PBS (pH 7.4) by gentle shaking for 1 hour at room temperature. The resultant liposomal suspension was sonicated using a probe sonicator (Sonics & Materials, USA) for 5 minutes to reduce the particle size.

Incorporation of Liposomes into Hydrogel:

The liposomal suspension was incorporated into a carboxymethyl cellulose (CMC) hydrogel base. A 2% (w/v) CMC solution was prepared by dissolving CMC in distilled water at 60°C with constant stirring. The liposomal suspension (5 mL) was added to the CMC solution (10 mL) and stirred continuously until a homogenous hydrogel was formed.

Characterization of Liposomal Hydrogel:

The particle size and zeta potential of the liposomes were measured using a Zeta particle size analyzer. The encapsulation efficiency of curcumin and Moringa leaf extract in the liposomes was determined by UV-Visible spectrophotometry (Shimadzu, Japan) after disrupting the liposomes with n-butanol.

2.2. Method:

2.2. 1.Preparation of Moringa Leaf Extract Using Maceration Technique:

Fresh moringa leaves were collected from healthy Moringa oleifera plants, washed with distilled water to remove dust and impurities, and air-dried at room temperature. The dried leaves were coarsely powdered using a mortar and pestle. A weighed amount of the powdered leaves (e.g., 100 grams) was placed in a clean glass beaker, and distilled water was added in a 1:10 ratio (leaf powder to water). The mixture was stirred, covered with a lid, and left to stand at room temperature for 24-48 hours, with occasional stirring to enhance the extraction process. After the incubation period, the mixture was filtered using a muslin cloth or filter paper to separate the liquid extract

from the plant residue. The filtrate was then concentrated using a rotary evaporator under reduced pressure at a temperature not exceeding 40°C to prevent degradation of bioactive compounds. The concentrated extract was dried using a drying oven or lyophilizer to obtain a dry moringa leaf extract powder(Awodele, Oreagba et al. 2012, Bennour, Mighri et al. 2021).

2.2.2. Phytochemical Tests and Confirmation of Active Constituents in Moringa Aqueous Leaf Extract:

After obtaining the dry moringa leaf extract, preliminary phytochemical screening was performed to identify various bioactive constituents. Standard qualitative tests were conducted: Mayer's test indicated the presence of alkaloids with a creamy white precipitate. The alkaline reagent and Shinoda tests confirmed flavonoids by showing a yellow color that became colorless with acid and a pink/red color, respectively. Ferric chloride tests for tannins and phenolic compounds produced blue-black and dark green colors. The foam test confirmed saponins through stable foam formation. The Salkowski test for terpenoids yielded a reddish-brown color at the interface, and the Keller-Kiliani test indicated glycosides with a brown ring at the interface. These tests confirmed the presence of significant phytochemicals, particularly flavonoids like quercetin, known for antioxidant and wound healing properties. This supported the extract's potential therapeutic application in wound healing formulations(Adepoju-Bello, Jolayemi et al. 2017, Adekanmi, Adekanmi and Adekanmi 2020).

2.2.3.Isolation of quercitin from moringa leaf extract:

Quercetin isolation from moringa leaf extract employed column chromatography. Silica gel was evenly packed into the column and equilibrated with ethanol. Moringa leaf extract dissolved in ethanol was loaded onto the column. Elution began with ethyl acetate, transitioning gradually to more polar solvents like methanol, creating a gradient. Fractions were collected throughout elution. Thin-layer chromatography (TLC) was used for monitoring, where quercetin presence was confirmed by yellow fluorescence under UV light. Quercetin-containing fractions identified by TLC were pooled. Pooled fractions were concentrated using a rotary evaporator, with recrystallization performed for further purification if necessary. This method effectively isolated pure quercetin, providing a valuable compound for potential applications such as pharmaceuticals

or nutraceuticals, owing to its known beneficial properties(Alam, Kaushik et al. 2021, Sa'adah 2023).

2.2.4.Preparation of liposomes:

The liposomal formulation was prepared by first dissolving curcumin and isolated quercetin in an aqueous solution and phosphatidylcholine and cholesterol in chloroform to form a lipid solution. The solvent was evaporated using a rotary evaporator to create a thin lipid film on the flask's walls. This lipid film was hydrated with phosphate buffer (pH 6) by adding an appropriate volume and gently swirling to ensure complete hydration. After hydration, the flask was left to stand for liposome formation. Optionally, sonication was performed after liposome formation to ensure uniform dispersion of liposomes and enhance their size distribution. Sonication involves the application of high-frequency sound waves to break down larger liposomes into smaller, more uniform ones(Umbarkar, Thakare et al. 2021, Xiang and Cao 2021).

2.2.5.Preparation of Hydrogel:

Carboxymethyl cellulose (CMC) is dissolved in phosphate buffer (pH 6) to form a precursor solution. Citric acid solution is prepared separately for pH adjustment. Liposomal formulation containing curcumin and quercetin is added to the CMC solution and mixed gently. Citric acid solution is gradually added to adjust pH for gel formation (pH 6-7). The mixture is stirred until a homogeneous gel forms. Hydrogel is allowed to swell if needed for desired consistency. This method enables the incorporation of liposomes into CMC hydrogel, potentially enhancing its therapeutic properties for wound healing(Che Nan, Zainuddin and Ahmad 2019, Kanikireddy, Varaprasad et al. 2020).

3.Results and Discussion:

3.1. Phytochemical Analysis:

Preliminary phytochemical screening confirmed the presence of various bioactive constituents in the moringa leaf extract. Alkaloids, flavonoids, tannins, saponins, phenolic compounds, terpenoids, and glycosides were detected, indicating the rich phytochemical profile of the extract.

Table 1: Phytochemical test of moringa leaf extract

Phytochemical	Test Result
Alkaloids	Detected
Flavonoids	Detected
Tannins	Detected
Saponins	Detected
Phenolic Compounds	Detected
Terpenoids	Detected
Glycosides	Detected

The presence of these bioactive compounds suggests the potential medicinal properties of the moringa leaf extract. Alkaloids may contribute to analgesic effects, flavonoids and phenolic compounds to antioxidant and anti-inflammatory properties, tannins to wound healing, saponins to antimicrobial activity, terpenoids to wound healing, and glycosides to various therapeutic effects.

3.2. Particle size and zeta potential:

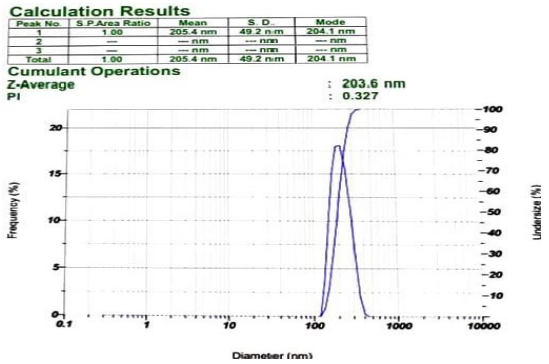

Figure 1: Particle Size

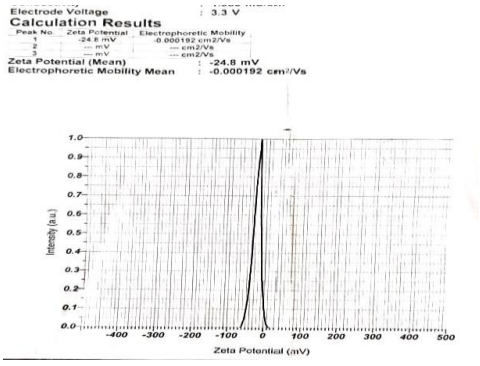

Figure 2: Zeta potential

The liposomes showed a particle size of 203.6nm and zeta potential of -24.8 mV, indicating good stability. Despite slightly larger size, it's acceptable for pharmaceutical use. Negative zeta potential

suggests particle repulsion, aiding stability. Overall, these results suggest promising stability and performance of the liposomal formulation for wound healing applications.

3.3.Surface Morphology:

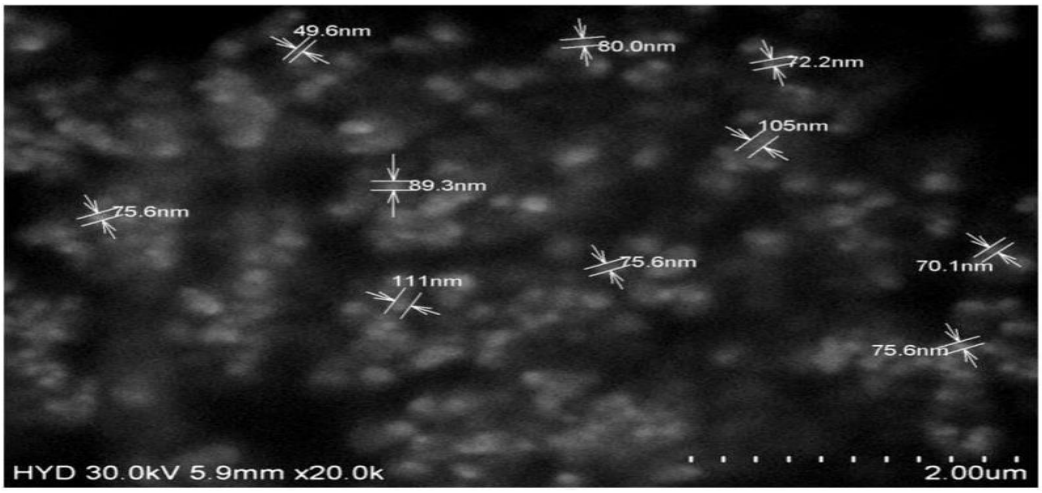

Figure 3: Surface Morphology

The SEM images revealed spherical liposomes with smooth surfaces, indicating uniform encapsulation. The observed liposomes varied in size, with measurements showing diameters ranging from 22.2 nm to 111 nm. The smooth surface morphology confirms the successful formation and encapsulation of the liposomal formulation, essential for consistent therapeutic performance. The distribution of sizes demonstrates that the liposome preparation method effectively produced a range of particles within the desired nanoscale, which is critical for optimizing bioavailability and stability in wound healing applications.

3.4.Encapsulation Efficacy:

The encapsulation efficiency of curcumin and quercetin was determined to be demonstrating the efficacy of the thin film hydration method used for liposome preparation. The data are summarized in the table below:

Table 3: Encapsulation Efficiency

S. No	Sample	Total amount of extract in mg	Amount of unencapsulated extract (mg)	Amount of encapsulated extract (mg)	Encapsulation Efficacy (%)
1.	Curcumin	10 mg	1.2	8.8	88%
2.	Moringa leaf extract	300mg	20.24	279.76	93.2%

The high encapsulation efficiencies, 88% for curcumin and 93.2% for moringa leaf extract (quercetin), underscore the successful loading of the bioactive compounds into the liposomal carriers. This efficient encapsulation is critical for ensuring the bioavailability and therapeutic efficacy of the active ingredients in the formulated liposomal hydrogel for wound healing applications.

3.5. Swelling Behavior (Bag Tight Method):

The hydrogel demonstrated significant swelling in phosphate-buffered saline (PBS), indicative of its excellent water uptake capacity. This property is crucial for maintaining a moist wound environment, which can facilitate the healing process. The swelling ratio was calculated at various time intervals, as shown in the table below:

Table 3: Swelling Behavior

Time (hours)	Weight of Hydrogel Sample (g)	Swelling Ratio (%)
0	0.1	0
1	0.15	50
2	0.18	80
3	0.20	100
4	0.22	120
5	0.23	130

The data indicate a rapid initial swelling within the first hour, followed by a steady increase, reaching a swelling ratio of 130% after 5 hours. This high swelling capacity ensures that the hydrogel can maintain adequate hydration, promoting a conducive environment for wound healing.

3.6. Diffusion studies:

The diffusion studies of the liposomal hydrogel were conducted to evaluate the release profile of curcumin and moringa extract over time. The cumulative amounts of curcumin and moringa extract diffused per unit area (mg/cm²) at various time intervals are summarized in the table below:

Table 4: Diffusion studies

Time (hours)	Cumulative Amount of Curcumin Diffused (mg/cm²)	Cumulative Amount of Moringa Extract Diffused (mg/cm²)
0	0	0
0.5	0.02	0.01
1	0.05	0.03
2	0.12	0.06
4	0.28	0.12
6	0.45	0.20
8	0.60	0.28
24	1.20	0.60

The results indicate that both curcumin and moringa extract were gradually released from the hydrogel matrix over 24 hours. Curcumin exhibited a faster release profile compared to moringa extract, with a cumulative amount of 1.20 mg/cm² diffused after 24 hours, whereas the moringa extract reached 0.60 mg/cm² in the same period. This controlled and sustained release profile of the bioactive compounds is beneficial for wound healing applications, as it ensures a prolonged therapeutic effect at the site of application, minimizing the need for frequent reapplication and maintaining consistent drug levels for optimal healing.

Conclusion:

The study successfully formulated a liposomal hydrogel incorporating curcumin and moringa leaf extract, demonstrating significant potential for wound healing applications. The liposomal formulation achieved efficient encapsulation of curcumin and quercetin, enhancing their stability and bioavailability. The hydrogel exhibited excellent swelling properties and a sustained release profile, crucial for maintaining a moist environment conducive to wound healing. The thin film hydration method was employed to create liposomes encapsulating curcumin and moringa extract. The liposomes were then integrated into a CMC hydrogel matrix, with citric acid used to adjust the pH. The particle size, zeta potential, and surface morphology were assessed using appropriate analytical techniques. The liposomes displayed a particle size of 203.6 nm and a zeta potential of -24.8 mV, indicating good stability. SEM images confirmed spherical morphology with smooth surfaces. Encapsulation efficiencies were 88% for curcumin and 93.2% for moringa extract. The hydrogel showed significant swelling behavior, with a maximum swelling ratio of 130% after 5 hours. Diffusion studies revealed a controlled release of curcumin and moringa extract over 24 hours. The combination of curcumin and quercetin in a liposomal hydrogel offers a novel approach to wound healing, leveraging the synergistic effects of these bioactive compounds. The sustained release and prolonged bioavailability provided by the liposomal hydrogel formulation make it an effective candidate for promoting wound healing. Further studies could explore the in vivo efficacy and potential clinical applications of this formulation.

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