Development and Evaluation of a Novel Microsphere Loaded Cream of *Tectona grandis* for Burn Scar Healing

M. Mumtaj Begum*, M. L. Indhumathi, E. Anbuselvam, E. Jegadeeswaran, B. Swathi Sree, B. Asfauk Ahamed, K. Veeramani, M. Afeefu Rahman

Department of Pharmaceutics, Sree Abirami College of Pharmacy, Eachanari, Coimbatore – 641 021, Tamil Nadu, India.

***Corresponding Author**

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ABSTRACT

Burn scars remain a significant challenge due to prolonged healing time. The present work focuses on the development and evaluation of a novel microsphere-loaded topical cream containing Tectona grandis extract for enhanced burn scar healing. Tectona grandis, known for its anti-inflammatory, antioxidant, and wound-healing properties, was selected as the active herbal component. Microspheres encapsulating the Tectona grandis leaf extract were prepared by Ionic gelation method using an appropriate polymeric system to achieve sustained drug release. The prepared microspheres were characterized for particle size, morphology, entrapment efficiency, swelling Index. Subsequently, the optimized microspheres F3 were incorporated into w/o cream base and evaluated for physicochemical properties such as pH, viscosity, spreadability, and extrudability. The formulated Tectona grandis microspheres loaded cream was subjected to Anti-oxidant activity with a maximum inhibition of approximately 78% at 100 µg/mL, comparable to the standard antioxidant ascorbic acid and Wound Healing Activity which showed 69.17% of wound healing effect on L929 cells after 24hrs of incubation. The research work concludes that the developed microsphere-loaded Tectona grandis cream holds promising potential as an effective topical therapeutic system for burn scar management, offering sustained drug delivery, improved healing outcomes and may be recommended for further evaluation studies and clinical studies.

Keywords: *Tectona grandis*, Microspheres, Ionic gelation method, w/o cream base, Anti-oxidant activity, Wound Healing Activity

INTRODUCTION

Burn injuries remain a significant global health challenge, often resulting in prolonged healing, infection risk, and permanent scarring. Effective management of burn wounds requires not only rapid tissue regeneration but also minimization of scar formation to restore both functional and aesthetic integrity of the skin. Conventional topical therapies, including antimicrobial creams and synthetic agents, frequently show limitations such as inadequate penetration, systemic side effects, and poor patient compliance. These challenges highlight the need for innovative, safe, and more effective therapeutic strategies. In recent years, natural products have gained considerable attention in wound healing research due to their biocompatibility, minimal toxicity, and diverse pharmacological properties.

Tectona grandis

Teak, or *Tectona grandis*, is a member of the Lamiaceae family and is found in many tropical countries, such as Thailand, Myanmar, and India. This plant's many parts have historically been used to heal wounds, skin diseases, microbial infections, and inflammatory ailments. Important phytoconstituents with antioxidant, antibacterial, and anti-inflammatory properties include flavonoids, phenolic compounds, naphthoquinones, and glycosides found in *Tectona grandis* leaves. Despite its therapeutic potential, the clinical application of *Tectona grandis* extract is often limited by poor stability, low bioavailability, and inadequate skin retention. To overcome these limitations, advanced drug delivery systems such as microspheres have been explored [1].

Microspheres

Microspheres are small spherical Particles, with a diameter in the micro metre range (typically 1 μm – 1000 μm). Microspheres are polymeric carriers that offer controlled and sustained release of bioactive compounds, improved stability, and enhanced localization at the target site. Microspheres can be manufactured from various natural and synthetic materials. Glass microspheres, polymer microspheres, and ceramic microspheres are commercially available. Solid and Hollow microspheres vary widely in density and therefore used for different applications. Bioadhesive, magnetic, floating, radioactive, and polymeric microspheres are among the several kinds of microspheres. Because of their safety and biocompatibility, biodegradable polymeric microspheres are frequently utilized in medicinal compositions. Incorporating plant extracts into microsphere systems can significantly enhance their therapeutic efficacy by prolonging drug residence time and reducing dosing frequency [2,3].

The protective barrier is disrupted when burns or wounds cause damage to the skin, necessitating appropriate therapy to aid in healing. Numerous cells, including fibroblasts, keratinocytes, and immune cells, take part in tissue regeneration and repair during this process. By lowering oxidative stress, avoiding infection, and encouraging tissue regeneration, herbal compositions with antioxidant, antibacterial, and anti-inflammatory qualities can greatly enhance wound healing. Topical creams serve as an ideal vehicle for dermal drug delivery due to their ease of application, patient acceptability, and ability to maintain a moist environment conducive to wound healing. The integration of microsphere-loaded systems into w/o cream base further enhances drug delivery by combining the advantages of controlled release with effective skin hydration and penetration [4]. Additional advantages of microsphere-loaded creams include better stability, regulated medication release, and increased active chemical penetration. Herbal ingredients can greatly increase the stability and therapeutic efficacy of microsphere-based creams, making them appropriate for burn treatment and wound healing. Herbal antioxidants, primarily driven by polyphenols, flavonoids, and phenolic acids, protect cells from free radical damage and oxidative stress.

MATERIALS AND METHODS

Materials

***Tectona grandis* Leaves** – Herbal Gardens, Coimbatore, **Sodium alginate –** Nice Chemicals, Pvt. Ltd., **Calcium chloride –** Nice Chemicals, Pvt. Ltd., **Yellow soft paraffin wax –** Oxford Lab Fine Chem LLP, **Bees wax** – Molychem, Mumbai, **Liquid paraffin** – Spectrum reagent and Chemicals, Pvt. Ltd., **Span 20** – Suvidhinath Laboratories, **Propylene glycol** – Loba Chemie Pvt.Ltd

Methodology

Collection of *Tectona grandis* Leaves

Fresh leaves of *Tectona grandis* were collected and washed thoroughly with distilled water to remove dust and impurities. The leaves were washed several times in running water and were dried in shade, placed at room temperature for about 2-3 weeks. Shade drying helps preserve the active phytochemical constituents. The dried leaves were then pulverized using a mechanical grinder to obtain a coarse powder. The powdered material was stored in an airtight container to prevent moisture absorption and degradation [5].

Extraction of Leaves of *Tectona grandis*

The powdered sample was extracted by maceration in ethanol 70% at a 1:5 (w/v) ratio (1 g leaf powder per 5 mL solvent). Samples were placed in sealed amber bottles and kept at room temperature (28–30 °C) for 72 hours. Bottles were gently shaken twice daily (about 1 min per time) to improve solvent–solid contact. The process is repeated 3-4 times to obtain sufficient amount of extract [6,7]. The mixture was then filtered through Whatman No.1 filter paper and the filtrate was concentrated and dried at room temperature for evacuating the residuals of ethanol. The semisolid extracts were stored in airtight amber containers at 15°C until analysis.

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Preparation of *Tectona grandis* Microspheres

Microspheres of *Tectona grandis* were prepared using the ionic gelation method by dissolving 2% sodium alginate in 100 mL distilled water in four different ratios (1:0.2, 1:0.3, 1:0.4 & 1: 0.5) under continuous stirring, followed by addition of the plant extract and adjustment of pH to 5.5 using 10% NaOH. A 5% calcium chloride solution was separately prepared and maintained under gentle stirring. The drug–alginate mixture was added dropwise into the calcium chloride solution at a controlled rate, forming gelled microspheres through ionic crosslinking. The formed microspheres were filtered, washed with acetone, and dried in an oven at 35°C. Finally, the dried microspheres were cooled in a desiccator and stored in an airtight amber-colored container until further use [8,9].

Table 1: Composition of *Tectona grandis* Microspheres

Formulation	Drug	Sodium alginate (in gm)	Calcium chloride (%w/v)
F1	5gm	1 gm	5%
F2	5gm	1.5gm	5%
F3	**5gm**	**2gm**	**5%**
F4	5gm	2.5gm	5%

Figure 1: Formulation of *Tectona grandis* Microspheres

Evaluation of Microspheres

Physico-Chemical Parameters:

1. **Particle Size and Size Distribution:**

   Microspheres are commonly characterized using conventional light microscopy (LM), particle size analyzers, and scanning electron microscopy (SEM) to assess their size, shape, and surface morphology. Additionally, electron spectroscopy for chemical analysis (ESCA) is employed to determine the surface chemical composition of the microspheres.

2. **Drug Entrapment Efficiency:**

   It can be calculated by calculated using the following equation,

   % Entrapment Efficiency = Actual content / Theoretical content x 100

3. **Drug Loading Efficiency:**

   Indicates the amount of drug successfully incorporated into microspheres. It can be calculated by calculated using the following equation,

% of Drug Loading = Amount of drug in microspheres / Total wt. of microspheres ×100

4. Swelling Index:

The swelling of microspheres was conducted in phosphate buffer of pH 6.8. Their diameters were periodically measured by using a laser particle size distribution analyzer until they were decreased by erosion and dissolution. The percentage of swelling was determined at different time intervals by the difference between diameter of microspheres at time t (D_t) and initial time t = 0, (D_0) as calculated from the following equation and averaged from 3 determinations [10],

Percentage of Swelling = $D_t - D_0 \times 100$

5. Zeta Potential:

Zeta potential is the electrical charge present on the surface of microspheres when dispersed in a liquid medium. Measures surface charge and stability of microspheres in suspension [11]. Higher absolute value indicates better stability.

Preparation of *Tectona grandis* Microspheres Cream

The *Tectona grandis* microsphere-loaded cream was prepared by the fusion (water-in-oil) method. The oil phase (white soft paraffin, liquid paraffin, beeswax, Span-80) and aqueous phase (propylene glycol, methyl paraben in water) were separately heated to 70–75°C, with vitamin E and perfume added to the aqueous phase. The aqueous phase was slowly added to the oil phase with continuous stirring to form an emulsion and cooled to room temperature. Microspheres (0.5 g) were then incorporated and mixed uniformly for 20–30 minutes. The prepared cream was stored in airtight containers at 25 °C, protected from light and moisture [12,13].

Table 2: Composition of *Tectona grandis* Microspheres Cream

S. No.	Ingredients	Required Quantity (5 Gm)
1.	*Tectona grandis* Microspheres	0.5 gm
2.	White Soft Paraffin	1 gm
3.	Liquid Paraffin	0.75 gm
4.	Bees Wax	0.25 gm
5.	Span 80	0.1 gm
6.	Distilled Water	2 gm
7.	Propylene Glycol	0.15 gm
8.	Methyl Paraben	0.025 gm
9.	Vitamin E	0.025 gm
10.	Perfume	q.s.

Figure 2: Preparation of *Tectona grandis* Microspheres Cream

Evaluation of Cream

The prepared water-in-oil (w/o) cream containing microspheres of *Tectona grandis* is evaluated for different physico-chemical parameters to determine its stability, quality, and suitability for topical application.

- **Appearance**

  The prepared *Tectona grandis* Microspheres cream is observed visually for color, texture, and homogeneity. The cream appeared pale yellow, smooth, and homogeneous with no visible lumps or phase separation.

- **pH Determination**

  1 g of *Tectona grandis* Microspheres cream is dispersed in 10 ml of distilled water and allowed to stand for 2 hours. The pH is measured using a calibrated digital pH meter. The pH of the prepared *Tectona grandis* Microspheres Cream was found to be 6.5, which is suitable for skin application [14].

- **Spreadability**

  Spreadability (s) is determined by placing a 1gm of *Tectona grandis* Microspheres cream sample between two glass slides to create a sandwich-like layer and applying 200g weight on the upper slide and allowed it to settle for 60 seconds. Carefully the weight was removed and the diameter of the spread cream was measured in centimeters (cm) using a ruler. It was calculated by [15],

$$S = \frac{M \times L}{T}$$

  where,

  S = Spreadability
  M = Weight tied to upper slide
  L = Length moved by the glass slide
  T = Time taken

  The cream showed good spreadability, indicating ease of application on skin.

- **Homogeneity**

  The prepared *Tectona grandis* Microspheres cream is tested by pressing a small quantity between the thumb and index finger. The formulation showed uniform distribution of microspheres without grittiness.

- **Extrudability**

  The prepared *Tectona grandis* microspheres w/o based cream showed good extrudability, indicating that the cream can be easily removed from the collapsible tube and is suitable for topical application. It can be calculated by

Extrudability = Weight applied to tube / Area of orifice

***In-vitro* Antioxidant Activity of *Tectona grandis* Microspheres Cream**

Using Phospho Molybdenum Method total antioxidant activity was calculated by mixing 0.5ml of the sample with the 0.5ml of reaction mixture contain 0.6 M H_2SO_4, 28mM sodium phosphate and 4mM ammonium molybdate reagent. The solution was incubated at 50°C for 90 minutes with blank. After incubation the tubes were normalised into room temperature and the absorbance was read at 695nm using spectrophotometer (LT 291 labtronics microprocessor). Ascorbic acid was used a standard to calculate the mg/gm of the total antioxidant activity.

***In-vitro* Wound Healing Activity *Tectona grandis* Microspheres Cream**

Cells were cultured in DMEM with high glucose supplemented with 10% FBS until 70–80% confluence, then seeded in 12-well plates (0.25 million cells/well) and incubated for 24 h to form a monolayer. A scratch was made using a sterile 200 µL pipette tip to create a wound gap, followed by washing to remove detached cells. The cells were then treated with test compounds and incubated at 37°C with 5% CO_2 for 24–48 h. Images were captured at specific time intervals (0, 24, 48 h), and wound closure was analyzed using imaging software.

The percentage of wound healing was calculated using the formula:

% of Wound Healing = [(Initial area − Final area) / Initial area] × 100.

RESULTS AND DISCUSSION

Phytochemical screening confirmed the presence of several bioactive compounds including alkaloids, flavonoids, phenols, terpenoids, and saponins. The formulation of *Tectona grandis* Microspheres was carried out by Ionic Gelation Technique using polymer, Sodium alginate and cross-linking agent, Calcium chloride. Drug: polymer ratio (1:0.2, 1:0.3, 1:0.4 & 1: 0.5) was selected based on the literature. The physico-chemical evaluation results indicated that Sodium alginate (2 gm) may be suitable as for the formulation of *Tectona grandis* Microspheres.

Table 3: Physico-Chemical Parameters of *Tectona grandis* Microspheres

Parameters	**F1**	**F2**	**F3**	**F4**
Colour	Pale green	Pale green	Pale green	Pale green
Surface Texture & Shape	Slightly dry, Spherical	Slightly Smooth, Spherical	Smooth, Spherical	Smooth, Spherical
Physical state	Free-flowing dry powder	Free-flowing dry powder	Free-flowing dry powder	Free-flowing powder
Entrapment efficiency	0.720	0.751	0.820	0.792
Swelling Index	0.321	0.403	0.442	0.514

SEM Analysis

Scanning Electron Microscopy (SEM) analysis (ZEISS) was used to study the surface morphology, shape, and particle characteristics of the prepared microspheres of *Tectona grandis*. A small quantity of dried *Tectona grandis* microspheres was mounted on an aluminum stub using double-sided adhesive tape. The sample was then coated with a thin layer of gold using a sputter coater to make the surface electrically conductive. The coated sample was examined under a Scanning Electron Microscope at suitable magnifications. The size of the beads were measured at 5µm (10.0kV). The beads were discrete and spherical in shape.

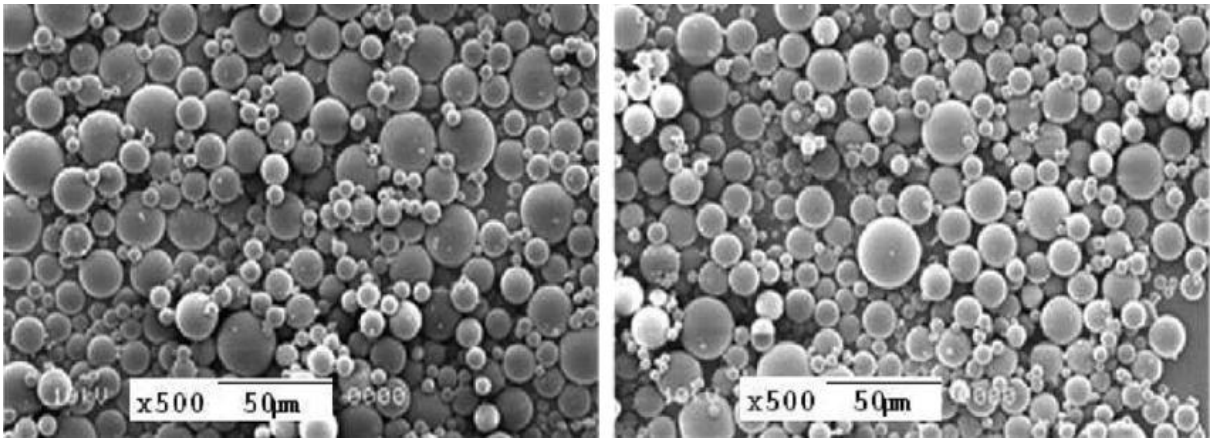

Figure 3: SEM image of *Tectona grandis* Microspheres

Physico-Chemical Evaluation of *Tectona grandis* Microspheres Cream

The prepared water-in-oil (w/o) cream containing microspheres of *Tectona grandis* was evaluated for different physico-chemical parameters to determine its stability, quality, and suitability for topical application.

Table 4: Physico-Chemical Parameters of *Tectona grandis* Microspheres Cream (F3)

Parameters	**F3**
Appearance	Pale yellow with no visible lumps or phase separation.
pH	6.5
Spreadability	35.1 g.cm/sec
Homogeneity	Smooth, No grittiness
Washability	Easily washable
Extrudability	Good

***In-vitro* Antioxidant Activity of *Tectona grandis* Microspheres Cream**

The *Tectona grandis* microspheres exhibited significant antioxidant activity, with a maximum inhibition of approximately 78% at 100 µg/mL, comparable to the standard antioxidant ascorbic acid. The sample details are given in the Table 5.

Table 5: Details of samples for the Anti-oxidant Activity

Sample	**OD**	**µg/ml**
Sample	0.257	258.6
STD (Ascorbic acid)	0.584	519.3

***In-vitro* Wound Healing Activity of *Tectona grandis* Microspheres Cream**

To measure the wound healing capacity on 3T3 cell lines, 1 test compound was used. The sample details are given in the Table 6. Percentage of Wound Healing can be calculated by

% of Wound Healing Scored= (Initial area-Final area)/Initial area x100

Overall the observed wound healing study results confirmed that the *Tectona grandis* Microsphere loaded cream was effectively promoted the wound healing effect of about 69.17% on L929 cells after 24hrs of incubation.

Table 6: Details of samples for the Wound Healing Activity

S. No.	**Sample Name**	**Sample code**	**Concentration**	**Cell line**
1	Sample	Sample	100ug/ml	L929
2	Cipladine	Cipladine	100ug/ml	L929

Table 7: Details of Untreated, Std control, Sample for measurement of wound area

3T3--Untreated Wound area measured Overlay			
Incubation	**Area**	**Initial Area-Final Area**	**% Wound Healing Scored**
0 hour	571071.23	0.00	0.00
24 hour	484819.36	86251.87	15.10
3T3-Std control Wound area measured Overlay			
Incubation	**Area**	**Initial Area-Final Area**	**% Wound Healing Scored**
0 hour	556394.03	0.00	0.00
24 hour	155561.22	400832.81	72.04

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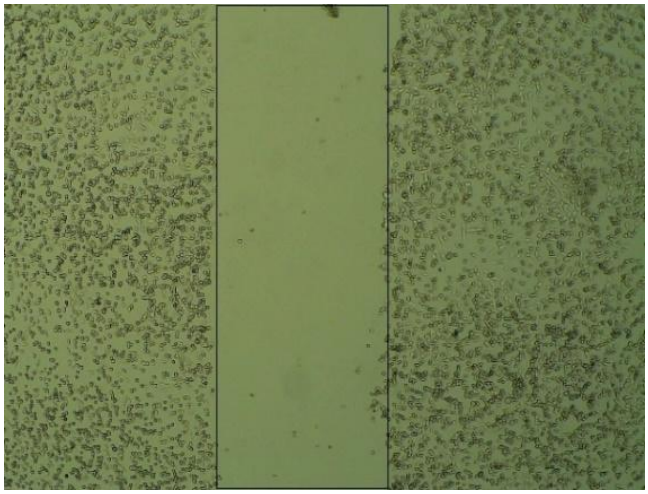
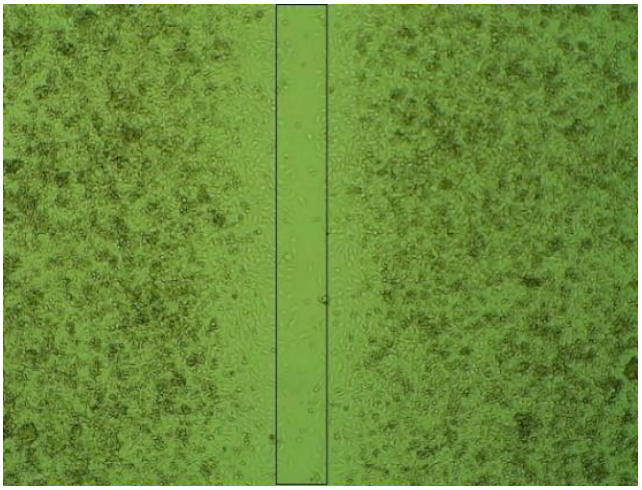
3T3- sample Wound area measured Overlay			
Incubation	**Area**	**Initial Area-Final Area**	**% Wound Healing Scored**
0 hour	564782.33	0.00	0.00
24 hour	174126.04	390656.29	69.17



Sample at 0 hours



Sample at 24 hours

Figure 4: Images of Sample for measurement of wound area at 0 hrs & at 24 hrs

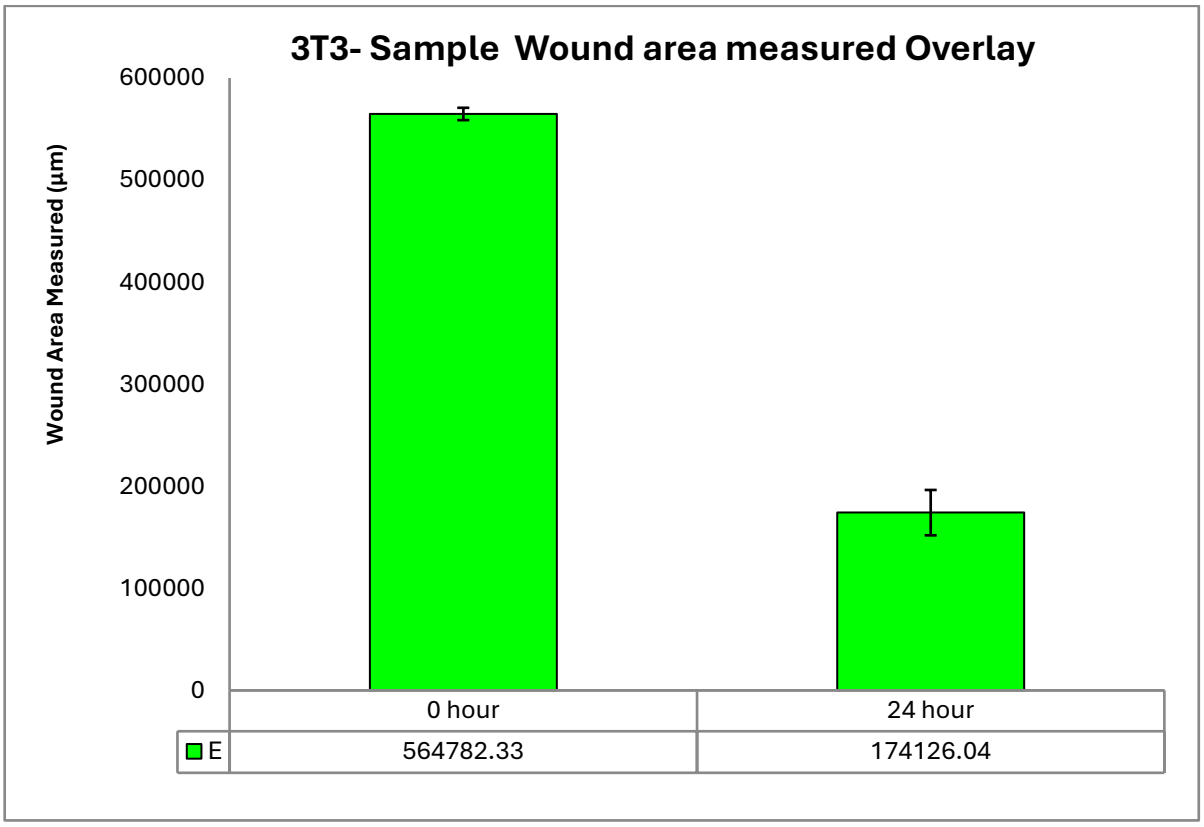


Figure 5: Details of Sample for measurement of wound area

SUMMARY AND CONCLUSION

The current work focused on developing *Tectona grandis* microspheres using the ionic gelation technique with sodium alginate as polymer and calcium chloride as cross-linking agent to achieve sustained drug release and good biocompatibility. The microspheres were evaluated for physicochemical properties, and the optimized

formulation (F3) was selected based on performance. These optimized microspheres were incorporated into a w/o topical cream using suitable excipients and further evaluated for stability and quality. The formulation showed significant antioxidant activity (~78% inhibition at 100 µg/mL) and promising wound healing activity (69.17% on L929 cells after 24 h). Overall, the developed microsphere-loaded cream demonstrates potential for sustained drug delivery and may be useful for burn scar healing, warranting further studies.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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