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Development and Evaluation of Liposomal Throat Spray Containing Tempuyung (*Sonchus Arvensis* L.) Leaf Extract for Antibacterial Activity Against *Streptococcus Pyogenes*

Rizka Sri Jayanty, Fajar Setiawan, Maya Nurul Rahma and Lusi Nurdianti*

Department of Pharmaceutics, Faculty of Pharmacy, Bakti Tunas Husada University, Tasikmalaya 46115, Indonesia

(*Corresponding author's e-mail: lusinurdianti@universitas-bth.ac.id)

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Abstract

Pharyngitis is a widely occurring infection of the upper respiratory tract, mainly caused by *Streptococcus pyogenes*, and continues to pose a substantial global health burden. Although *Sonchus arvensis* L. leaves exhibit promising antibacterial activity, their application is limited by poor stability and inadequate delivery to the target site. Therefore, this study aimed to develop a liposome-based throat spray formulation of tempuyung leaf extract (TLE-LipTS) to enhance antibacterial activity and stability. The extract was prepared through maceration with 96% ethanol as the solvent and subsequently encapsulated into liposomes using a heating method with soy lecithin and cholesterol. The liposomal system was characterized in terms of particle size, polydispersity index (PDI), and zeta potential, followed by evaluation of pH, viscosity, spreadability, antibacterial activity against *Streptococcus pyogenes*, and storage stability. The results showed that TLE-Lip had a particle size of 477.3 nm, a PDI of 0.7843, and a zeta potential of –11.27 mV, indicating a large unilamellar vesicle (LUV) system with relatively low stability. The throat spray formulations exhibited acceptable physicochemical properties, with pH values ranging from 7.01 - 7.46, viscosity between 40.80 - 116.33 cP, and spreadability within the optimal range of 5 - 7 cm. Antibacterial testing revealed that the highest concentration (F3) demonstrated the strongest activity, with an inhibition zone of 16.17 ± 0.38 mm, exceeding the positive control, and statistical analysis confirmed significant differences among formulations ($p < 0.05$). Stability studies indicated that storage at 4 °C better preserved physicochemical properties and antibacterial activity compared to room temperature. In conclusion, the liposomal throat spray formulation of tempuyung leaf extract enhances antibacterial activity and stability, suggesting its potential as an alternative therapeutic approach for the treatment of pharyngitis.

Keyword: *Sonchus arvensis L.*, Pharyngitis, Liposome, Throat spray, Antibacterial activity, *Streptococcus pyogenes*, Stability

Introduction

Pharyngitis is among the most frequently occurring infections of the upper respiratory tract and remains a major public health concern in Indonesia. This condition is predominantly caused by Streptococcus pyogenes. Globally, pharyngitis accounts for more than 700 million cases annually, with the highest incidence observed among school-aged children [1]. Globally, pharyngitis is a highly prevalent condition affecting individuals of all age groups and accounts for approximately 1% - 2% of total outpatient visits, indicating a significant burden on healthcare systems [2].

Phenol is used in medical practice for the treatment of pharyngitis and is available in Indonesia in the form of throat spray preparations. Formulations containing phenol as an active ingredient can provide temporary symptom relief by reducing pain and irritation of the throat mucosa. However, phenol is associated with several adverse effects, including mucosal irritation, toxicity, and the risk of systemic effects due to its corrosive nature. Furthermore, phenol may cause tissue

damage and can be potentially harmful if not used appropriately, thereby limiting its use in long-term therapy [3].

Sonchus arvensis L are considered a promising medicinal plant that has traditionally been used to manage various conditions, including as a diuretic, for kidney stones, gallstones, swelling, asthma, cholesterol reduction, bronchitis, hyperuricemia, as well as an antioxidant, and also possess antibacterial activity [4,5]. Tempuyung leaves (*Sonchus arvensis* L.) have been reported to exhibit strong antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. A study demonstrated that tempuyung leaf infusion at a concentration of 4 g/mL produced inhibition zone diameters of 12.23 mm against *Escherichia coli* and 7.88 mm against *Staphylococcus aureus*, which are categorized as moderate to strong antibacterial activity [5]. In addition, another study reported that tempuyung leaf extract showed significant antibacterial activity against *Streptococcus mutans*, minimum inhibitory concentration (MIC) value of 15.6 µg/mL, categorized as very strong [6].

Despite its promising antibacterial activity, the application of tempuyung leaf extract remains limited by issues related to stability and effective delivery to the target site. Bacteria are capable of forming biofilms composed of complex matrices, including lipid components, which hinder the penetration of plant extracts [7]. Therefore, a liposomal drug delivery system is required to enhance the penetration of active compounds into bacterial cells. Liposomes possess a phospholipid bilayer structure similar to biological membranes, enabling them to interact with biofilm matrices and bacterial cell membranes [8]. A previous study demonstrated that nanoliposomes containing Toluidine Blue O showed antibacterial effect against *Streptococcus mutans*, inhibition zone of 0.7 ± 0.2 mm at 1 µg/mL, compared to 1.2 ± 0.3 mm for the 2% positive control. Although the inhibition zone diameter was relatively small, these results still highlight the potential of liposomal systems to exert antibacterial effects even at low concentrations [9].

The selection of an appropriate delivery system is an important factor in therapeutic application, and throat spray can be utilized for the treatment of pharyngitis [10]. Throat spray is one of the preferred dosage forms for managing sore throat due to its ability to deliver active compounds directly to the target site while providing a soothing effect on the mucosal surface. This effect is not only attributed to the active ingredients but also to the role of excipients, particularly humectants such as glycerol, which play an essential role in maintaining throat moisture [11].

Therefore, this study aims to evaluate the antibacterial activity against *Streptococcus pyogenes*, the causative agent of pharyngitis, using tempuyung (*Sonchus arvensis* L.) leaf extract encapsulated in a liposomal system and formulated as a throat spray, as a potential alternative therapy for the treatment of pharyngitis.

Materials and methods

Material

Tempuyung leaves (*Sonchus arvensis* L.), ethanol 96% (Merck), soya lecithin (Indoplant), cholesterol (Sigma-Aldrich), buffer pH 7.4, phenol crystal commercial (cooling 5), glycerin (onemed), sorbitol (Merck), menthol (Merck), distilled water, bacteria *Streptococcus pyogenes* (ATCC 19615)*, Blood Agar Base* (OXOID), NaCl 0.9%, paper disc.

Method

Preparation of tempuyung leaf (Sonchus arvensis L.) extract

Fresh tempuyung (*Sonchus arvensis* L.) leaves (1 kg) were rinsed under running water to eliminate adhering impurities, then cut into small pieces and oven-dried at 40 °C until fully dried. The dried material was subsequently ground and sieved through mesh no. 60 to obtain a uniform particle size. Extraction process was performed by maceration using 96% ethanol as the solvent for 3×24 h with intermittent stirring. The mixture was then filtered through Whatman No. 1 filter paper, and the resulting filtrate was concentrated using a rotary evaporator to obtain a viscous tempuyung leaf extract [12].

Characterization of tempuyung leaf extract (TLE)

Characterization of tempuyung (*Sonchus arvensis* L.) leaf extract was carried out using several parameters, including moisture content, total ash content, loss on drying, water-soluble extractive value, and ethanol-soluble extractive value. Moisture content was measured

by toluene distillation, whereas total ash was determined by high-temperature incineration until a constant weight was reached. Loss on drying was measured by heating the extract at 105 °C until a constant weight was obtained. Meanwhile, the water-soluble and ethanol-soluble extractive values were determined by dissolving the extract in the respective solvents, followed by filtration, evaporation, and drying to constant weight.

Phytochemical screening was performed to identify the presence of secondary metabolites in the extract using standard qualitative methods. The extract was tested for several classes of compounds, including alkaloids (Bouchardat and Mayer reagents), flavonoids (Mg-HCl reaction), saponins (foam test after the addition of 2 N HCl), tannins and phenolic compounds ($FeCl_3$ and gelatin tests), monoterpenoids and sesquiterpenoids (anisaldehyde or vanillin-sulfuric acid), quinones (NaOH reaction), as well as steroids and triterpenoids (Liebermann-Burchard reaction). The presence of each compound group was indicated by characteristic color changes or the formation of precipitates [13].

Manufacturing of tempuyung leaf extract liposomes (TLE-Lip)

TLE-Lip were prepared using the heating method by dissolving soy lecithin (0.5 g) in distilled water at 60 °C under continuous stirring with a magnetic stirrer until a homogeneous solution was obtained, followed by the addition of cholesterol (0.05 g) and further stirring until completely mixed (Solution A). The tempuyung leaf extract was dissolved in phosphate buffer pH 7.4 and homogenized using a magnetic stirrer at 300 rpm and 60 °C for 30 min to obtain a homogeneous mixture (Solution B). Subsequently, Solution A was gradually added into Solution B under continuous stirring at the same conditions until a homogeneous liposomal suspension was formed. The suspension was then sonicated for 30 min at 60 °C to reduce vesicle size and achieve a more uniform particle distribution [14].

Table 1 Formulations of TLE-Lip.

Materials	Formulations (g)		Function
	F0	F1	
TLE	-	0.8	Active ingredient
Soya lecithin	0.5	0.5	Lipid phase
Cholesterol	0.05	0.05	Stabilizing agent
Aquadest ad	20 mL	20 mL	Solvent

Characterization of tempuyung leaf extract liposomes (TLE-Lip)

Particle size and polydispersity index

The particle size and polydispersity index of TLE-Lip were determined using the dynamic light scattering (DLS) technique with a particle size analyzer. Prior to measurement, the liposomal dispersion was diluted with phosphate buffer pH 7.4 to minimize multiple scattering effects and ensure accurate results. Approximately ± 1 mL of TLE-Lip was dispersed in distilled water, and the sample was then transferred into a flow cell cuvette for analysis [15].

Zeta potential

The zeta potential of the liposomes was measured using a particle size analyzer equipped with electrophoretic light scattering. Approximately ± 1 mL of TLE-Lip was placed into a cuvette and analyzed using a zetasizer probe. The electrophoretic mobility of the particles was measured to calculate the zeta potential, which was used to evaluate the surface charge and stability of the liposomal system [16].

Manufacturing of throat spray tempuyung leaf extract liposomes (TLE-LipTS)

The tempuyung (*Sonchus arvensis* L.) leaf extract liposome throat spray (TLE-LipTS) was prepared by first mixing glycerin and sorbitol until a homogeneous mixture was obtained. Menthol, previously dissolved in 96% ethanol, was then added to sodium benzoate that had been dissolved in distilled water, followed by gentle stirring until uniformly mixed. The liposomal suspension of tempuyung leaf extract was subsequently incorporated into the mixture and stirred until

homogeneous. The final volume was adjusted with distilled water up to 100 mL [17].

Table 2 Formulations of TLE-LipTS.

Materials	Formulations % (b/v)				Function
	F0	F1	F2	F3	
TLE-Lip	-	5	7,5	10	Active Ingredient
Glycerin	5	5	5	5	Emollien/Humectant
HPMC	0,1	0,1	0,1	0,1	Thickening Agent
Sorbitol	10	10	10	10	Sweetener
Sodium Benzoate	0,1	0,1	0,1	0,1	Preservative
Menthol	0,05	0,05	0,05	0,05	Flavoring Agent
Distilled Water ad	100	100	100	100	Solvent

Evaluation of throat spray tempuyung leaf extract liposomes (TLE-LipTS)

pH measurement

The pH of TLE-LipTS was measured using a digital pH meter. Prior to measurement, the instrument was calibrated using standard buffer solutions at pH 2.0, 7.0, and 10.0 to ensure accuracy. The electrode was then immersed in the sample, and the pH value of the formulation was recorded.

Viscosity measurement

Viscosity was measured using a Brookfield viscometer with spindle number 62 immersed into the sample. The instrument was operated at a speed of 30 - 100 rpm [18].

Spreadability

The spray formulation was applied onto a glass plate from a distance of 5 cm, and the spreadability was subsequently measured. An optimal spreadability range is between 5 - 7 cm [17].

Antibacterial activity test

The antibacterial activity of TLE-LipTS formulations was evaluated against *Streptococcus pyogenes* using the disk diffusion method. The bacterial suspension was prepared and inoculated onto the surface of blood agar media, followed by the application of samples using paper discs. The plates were incubated at 37 °C for 24 h, after which antibacterial activity was assessed by measuring the diameter of the inhibition zones surrounding each sample [19].

Storage stability test

TLE-LipTS formulations were stored at room temperature and at 4 °C for 30 days. After storage TLE-LipTS was evaluated for pH, viscosity, spreadability, and antibacterial activity [20].

Data analysis

The results from characterization and evaluation were presented as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA with SPSS software to assess differences between groups [21]. Graphical representation of the data was used to evaluate the physicochemical characteristics and antibacterial performance of the liposomal formulations.

Results and discussion

Preparation of tempuyung leaf (*Sonchus arvensis* L.) extract

Extraction of tempuyung (*Sonchus arvensis* L.) leaves was carried out using the maceration method with 96% ethanol as the solvent. This method was selected due to its simplicity and its ability to preserve thermolabile compounds such as flavonoids and phenolic constituents, as it does not involve direct heating, thereby minimizing degradation of active compounds [12]. The use of 96% ethanol is considered appropriate due to its amphiphilic nature, allowing it to

dissolve a wide range of hydrophilic and lipophilic compounds. This property enables ethanol to extract various bioactive compounds such as phenolics, lipids, and terpenoids, making it widely used in natural product extraction. Furthermore, an extraction duration of 3×24 h provides sufficient contact time between the solvent and plant material, facilitating optimal diffusion of active compounds and maximizing extraction yield [22].

Filtration using Whatman filter paper was performed to separate the extract from plant residues, resulting in a clear filtrate for subsequent concentration [23]. The extract was subsequently concentrated using a rotary evaporator at 50 °C under reduced pressure, allowing efficient solvent removal while preserving heat-sensitive compounds. This condition allows evaporation at a lower boiling point, thereby maintaining the stability of the bioactive constituents [24].

The extraction process yielded a thick extract with a rendement of 13.84% from 300 g of dried simplicia. This value indicates that *Sonchus arvensis* leaves contain a considerable amount of ethanol-soluble compounds, particularly flavonoids and phenolic constituents. The relatively high yield suggests that the extraction method and solvent used were effective; however, it may still be influenced by factors such as plant origin, drying conditions, particle size, and solvent polarity [25].

Characterization of tempuyung leaf extract (TLE)

Characterization of tempuyung leaf extract (*Sonchus arvensis* L.) was performed to ensure its quality, stability, and suitability as a pharmaceutical active ingredient. Non-specific parameters, including moisture content, total ash content, extractive values, and loss on drying, were evaluated as part of standard quality control to ensure the consistency and safety of the extract prior to formulation [26].

Table 3 Characterization results of Tempuyung Leaf Extract (TLE).

Parameter	Results
Moisture Content	2%
Total Ash Content	10%
Water-Soluble Extractive Value	50.5%
ethanol-soluble extractive value	86.8%
Loss on Drying	2%

The moisture content and loss on drying of tempuyung leaf extract (TLE) were both 2%, which are well below the acceptable limits (<12.5% and <30%). These low values indicate minimal water and volatile content in the extract, which can inhibit microbial growth and enhance stability during storage. Extracts with low moisture content are generally more resistant to degradation and have a longer shelf life, making them more suitable for further formulation processes [26].

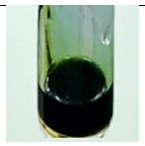
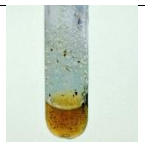
The total ash content of the extract was 10%, which complies with the standard requirement (<13.1%). This parameter reflects the amount of inorganic constituents present in the extract and serves as an indicator of purity. A controlled ash value suggests that contamination from external sources such as soil, dust, or other inorganic residues during preparation and processing is minimal [26].

Extractive values provide insight into the solubility of bioactive compounds in specific solvents and indirectly reflect the chemical composition of the extract. The water-soluble extractive value of TLE was 50.5%, exceeding the minimum requirement (>34.5%), indicating a high content of polar compounds such as flavonoids and phenolic constituents. Meanwhile, the ethanol-soluble extractive value was 86.8%, far above the standard (>9%), suggesting that the extract is rich in semi-polar to non-polar compounds, including flavonoids, terpenoids, and other secondary metabolites [27]. These results indicate that ethanol is an effective solvent for extracting a wide range of bioactive compounds from *Sonchus arvensis* leaves.

Table 4 shows the presence of several important secondary metabolites in TLE, including alkaloids, flavonoids, polyphenols, and steroids. These

compounds exhibit notable pharmacological properties, particularly antibacterial activity. Flavonoids and polyphenols are capable of disrupting bacterial cell membranes and inhibiting key enzymatic processes, while alkaloids can interfere with DNA replication and protein synthesis [28]. The presence of these compounds further supports the potential of tempuyung leaf extract as an antibacterial agent.

Table 4 Results screening pytochemical of tempuyung leaf extract (TLE).

Parameter	Results	
Alkaloids	Formation of an orange precipitate (+)	
Flavonoids	Color change to yellow (+)	
Saponins	No foam formation (-)	
Polyphenols	Color change to dark greenish-black (+)	
Steroids	Color change to bluish-green (+)	
Tannins	No precipitate formed (-)	

However, saponins and tannins were not detected in this study. This finding differs from previous reports that identified these compounds in *Sonchus arvensis* extracts. These variations may be explained by multiple factors, such as environmental conditions, the plant's geographical origin, harvesting period, extraction techniques, solvent polarity, and the sensitivity of the qualitative phytochemical screening methods used. Phytochemical composition is highly dependent on biosynthetic pathways, plant part variability, and external conditions during growth and processing. These factors are known to significantly influence the chemical profile of plant extracts [27,28].

Overall, the characterization results indicate that TLE meets the required quality standards and contains a variety of bioactive compounds. Its favorable physicochemical properties and phytochemical composition suggest strong potential for further development, particularly in advanced drug delivery systems such as liposomes to enhance stability and antibacterial effectiveness.

Manufacturing of tempuyung leaf extract liposomes (TLE-Lip)

Figure 1 illustrates the preparation of tempuyung leaf extract liposomes (TLE-Lip) using the heating method. This method offers advantages as it does not involve the use of organic solvents, thereby reducing the risk of residual solvent toxicity and making it safer for thermolabile active compounds [14]. In this formulation, soy lecithin acts as the primary phospholipid component forming the liposomal bilayer

structure, while cholesterol functions as a stabilizing agent that enhances membrane rigidity, maintains vesicle integrity, and prevents leakage of encapsulated active compounds [29].

The use of a temperature of 60 °C is intended to ensure that phospholipids are above their phase transition temperature, thereby increasing membrane fluidity and facilitating the formation of a stable bilayer structure. Homogenization using a magnetic stirrer at 300 rpm provides sufficient energy to achieve uniform dispersion without damaging the vesicular structure [30].

Furthermore, the use of phosphate buffer at pH 7.4 not only aims to mimic physiological conditions but also plays a crucial role in maintaining the aqueous environment required for effective drug encapsulation within the liposomal system. Liposomes are formed by entrapping an aqueous phase within a phospholipid bilayer, and thus the physicochemical properties of the medium, including pH, significantly influence encapsulation efficiency and vesicle stability. The pH condition also affects the interaction between encapsulated compounds and the lipid bilayer, which in turn influences drug retention and distribution within the vesicles. Therefore, a near-neutral pH is essential to preserve the structural integrity of liposomes and to support the optimal performance of the delivery system [31].

The resulting suspension was subsequently sonicated for 30 min at 60 °C to reduce particle size and improve the uniformity of the liposomal distribution. The sonication process facilitates the transformation of multilamellar vesicles into smaller vesicles with a more homogeneous size distribution, thereby enhancing stability and drug delivery efficiency [32].

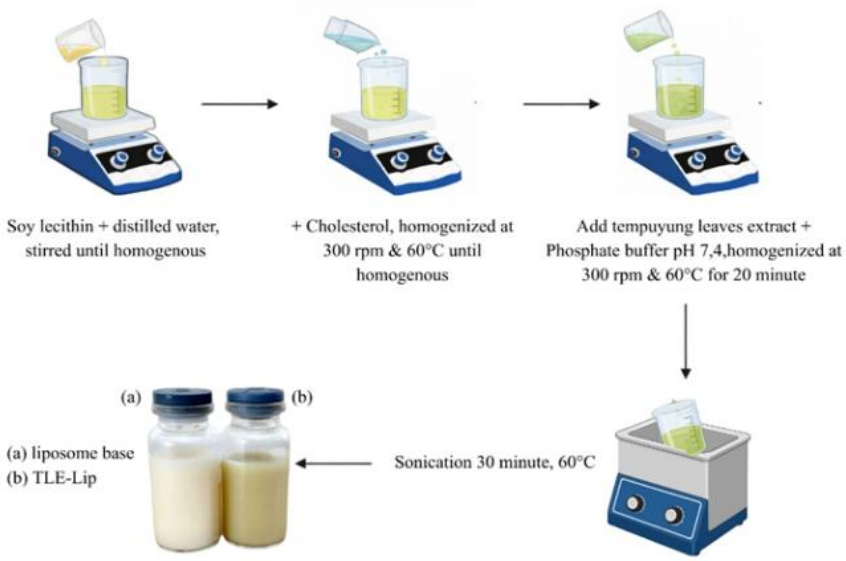


Figure 1 Tempuyung Leaf Extract Liposomal (TLE-Lip) Formulation Procedure.

Table 5 Characterization Results of Particle Size, PDI, and Zeta Potential of TLE-Lip.

PSA value (nm)	Polydispersity Index (PI)	Zeta potential (mV)
477.3 ± 7.41	0.7843 ± 0.02	−11.27 ± 0.87

Characterization of TLE-Lip

Liposomal characterization was performed by measuring particle size, polydispersity index (PDI), and zeta potential using a particle size analyzer (PSA).

The particle size of TLE-Lip was 477.3 nm, which falls within the category of large unilamellar vesicles (LUV) known for their high encapsulation capacity and structural stability. This size is relatively larger compared to previous studies, although similar preparation procedures were employed. Such differences may be attributed to formulation and processing factors, particularly sonication parameters

and mixing temperature, which are known to significantly influence liposome particle size [14]. The obtained PDI value of 0.7843 indicates a non-uniform particle size distribution (PDI >0.3), likely due to uneven distribution of sonication energy, resulting in heterogeneous vesicle sizes [32].

Meanwhile, the zeta potential value of −11.27 mV falls below the ideal stability threshold (± 30 mV), indicating low electrostatic stability. This condition may be influenced by the use of neutral phospholipids such as soy lecithin and cholesterol, leading to reduced repulsive forces between particles and a higher tendency for [32].

The results of this study are generally consistent with those reported by Ahmed *et al*., where both studies employed conventional liposome preparation methods based on soy lecithin and produced liposomes within the large unilamellar vesicle (LUV) size range with negative zeta potential. However, differences in the obtained particle size may be attributed to variations in preparation methods and processing conditions [33].

Manufacturing of throat spray tempuyung leaf extract liposomes (TLE-LipTS)

The formulation of TLE-LipTS was designed by considering the functional roles of each component to ensure stability, effectiveness, and patient comfort. Hydroxypropyl methylcellulose (HPMC) was used as a viscosity enhancing agent to improve retention of the formulation on the mucosal surface, where its dispersion in hot water facilitates polymer hydration and the formation of a homogeneous system [34]. The addition of humectants such as glycerin and sorbitol plays an important role in maintaining moisture and preventing dryness of the mucosal surface. Glycerol exhibits hygroscopic properties that enable it to attract and retain water, thereby providing moisturizing, lubricating, and demulcent effects that help form a protective layer and reduce mucosal irritation [11].

Sodium benzoate was incorporated as a preservative to inhibit microbial growth in the aqueous system, thereby ensuring product safety and stability during storage. Meanwhile, menthol functions as a sensory agent that provides a cooling and soothing effect, enhancing patient comfort and the perceived effectiveness of the formulation. The dissolution of menthol with other components also facilitates uniform distribution within the system, prevents crystallization, and maintains formulation clarity [35].

The presence of the extract in liposomal form contributes to increased color intensity, particularly at higher concentrations, while still maintaining appropriate physical characteristics for throat spray application. The use of glycerin and sorbitol as humectants and stabilizing agents supports the formation of a homogeneous system, improves viscosity, and enhances comfort during application in the oral cavity [11]. In addition, menthol provides a cooling sensation that further improves patient comfort during use.

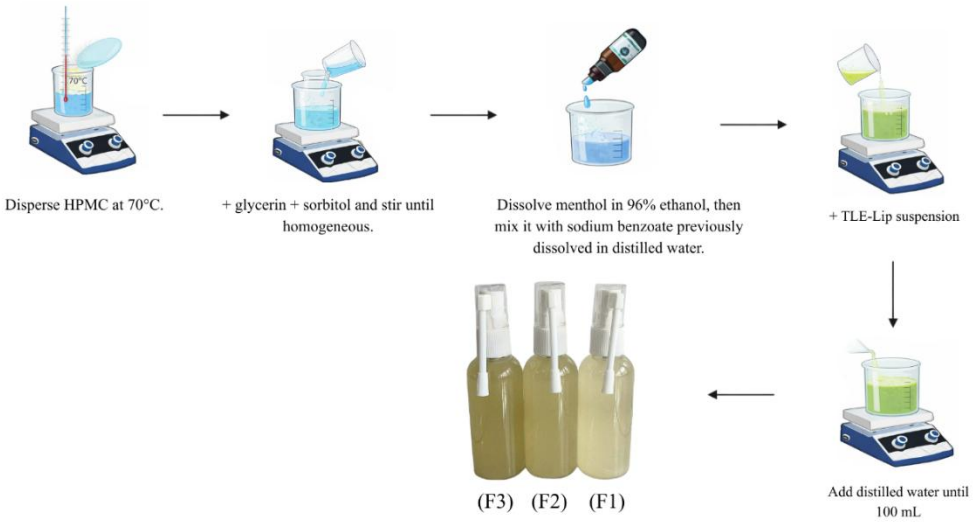


Figure 2 Throat Spray Tempuyung Leaf Extract Liposomal Formulation Procedure.

Evaluation of throat spray tempuyung leaf extract liposomes (TLE-LipTS)

The evaluation results of TLE-LipTS showed that all formulations met the general requirements for throat spray preparations. Organoleptically, the formulations appeared as liquids with a characteristic menthol aroma, a slightly sweet taste, and color variations ranging from clear (F0), pale yellow (F1), to green at higher concentrations (F2 and F3). These color differences indicate the influence of the liposomal extract concentration used in the formulations.

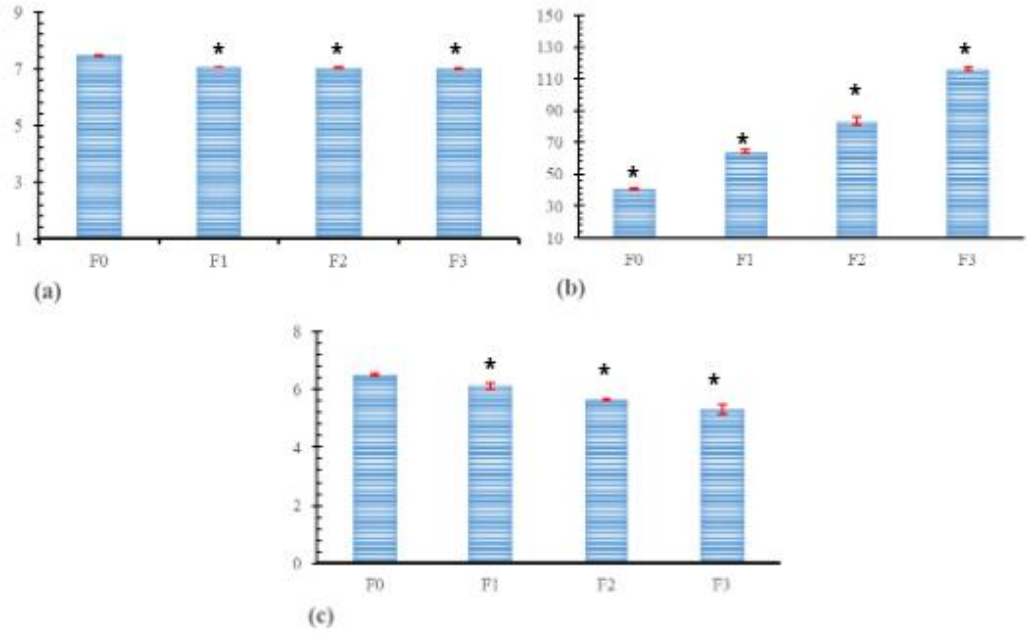


Figure 3 Evaluated of Throat Spray Tempuyung Leaf Extract Liposomes (TLE-LipTS) (a) pH Measurement; (b) Viscosity Measurement; (c) Spreadability. Data are presented as mean ± standard deviation (n = 3); * indicates statistical significance ($p < 0.05$), while # indicates no significant difference ($p > 0.05$) compared with F0.

The pH evaluation results showed that all TLE-LipTS formulations were within the acceptable range for throat spray preparations (6.5 - 7.5), indicating good compatibility with the oral mucosa and minimal irritation potential [36]. he measured pH values were 7.46 ± 0.01 (F0), 7.04 ± 0.00 (F1), 7.03 ± 0.01 (F2), and 7.01 ± 0.01 (F3), which are close to neutral and important for maintaining user comfort and preventing mucosal damage. These variations may be influenced by the presence of bioactive compounds in tempuyung leaf extract, such as flavonoids and phenolic compounds, which exhibit weakly acidic properties due to hydroxyl groups capable of deprotonation with pKa values near physiological pH. This behavior is affected by the number and position of hydroxyl groups within their molecular structures [37].

The viscosity evaluation results showed that all formulations possessed suitable consistency for spray application. The measured viscosity values were 40.80 ± 0.33 cP (F0), 64.00 ± 1.13 cP (F1), 83.67 ± 2.62 cP (F2), and 116.33 ± 0.94 cP (F3). Viscosity plays an important role in determining sprayability as well as the ability of the formulation to remain on the mucosal surface. In this formulation, glycerin as a humectant and HPMC as a thickening agent contributed to maintaining optimal viscosity and improving system stability. An appropriate viscosity ensures that the formulation is not too fluid while still allowing efficient spraying [11].

The spray spreadability test showed that all formulations were within the optimal range of 5 - 7 cm, indicating good dispersion characteristics [38]. The measured spreadability values were 6.51 ± 0.05 cm (F0), 6.13 ± 0.12 cm (F1), 5.64 ± 0.03 cm (F2), and 5.30 ± 0.16 cm (F3). These results indicate that the formulation is capable of spreading evenly on the mucosal surface, thereby supporting uniform distribution of active

compounds. Adequate spreadability also enhances the contact between liposomes and the target tissue, which may improve the delivery efficiency and antibacterial activity of the encapsulated tempuyung leaf extract.

Statistical analysis revealed significant differences among formulations in terms of pH, viscosity, and spreadability ($p < 0.05$). This indicates that variations in the concentration of liposome-encapsulated tempuyung leaf extract influenced the physicochemical properties of the formulations.

Antibacterial activity test

The TLE-LipTS formulations exhibited antibacterial activity against *Streptococcus pyogenes* in F1 - F3, whereas spray base (F0) showed no inhibition zone (0 mm), confirming the absence of antibacterial activity in the formulation without the extract.

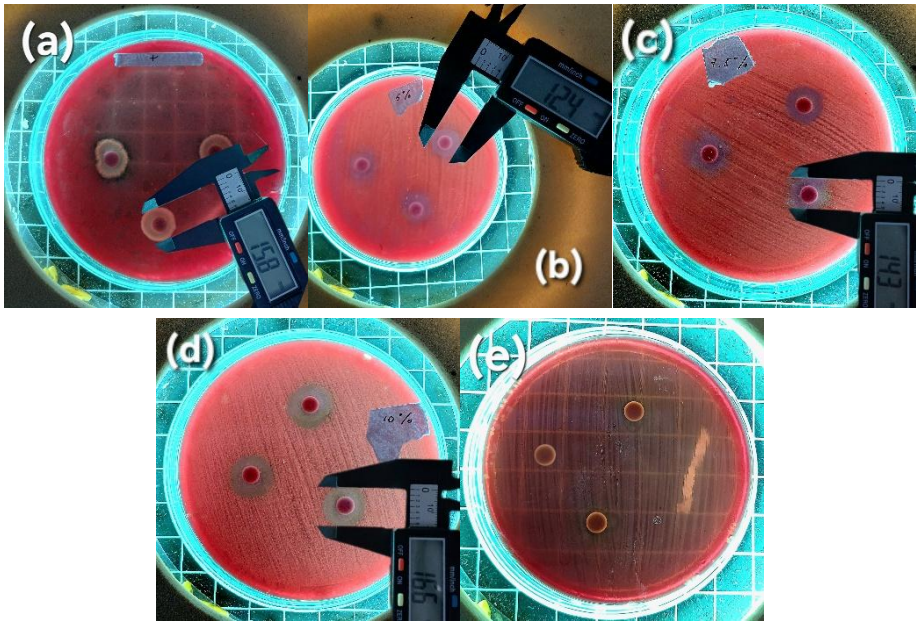


Figure 4 Inhibition Zone (mm) of (a) phenol commercial; (b) F1; (c) F2; (d) F3; (e) F0.

Among all tested formulations, F3 exhibited the largest inhibition zone diameter (16.17 ± 0.38 mm), followed by F2 (14.3 ± 0.60 mm) and F1 (12.3 ± 0.44 mm). The positive control showed an inhibition zone of 15.3 ± 0.42 mm, which falls within the strong category. Notably, F3 demonstrated slightly higher antibacterial activity compared to the positive control, indicating the strong potential of the liposomal formulation.

These results also demonstrate a clear relationship between increasing extract concentration and enhanced antibacterial activity. Statistical analysis further confirmed that the antibacterial activity differed significantly among treatment groups ($p < 0.05$; sig. 0.000).

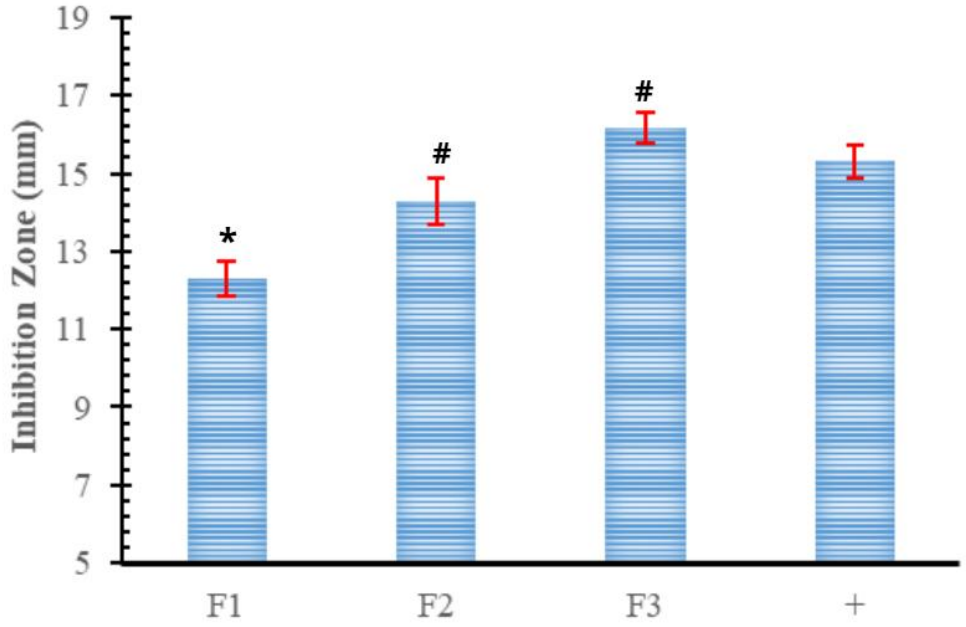


Figure 5 Inhibition Zone TLE-LipTS. Data are presented as mean ± standard deviation (n = 3); * significance level of $p < 0.05$ and # insignificance level of $p > 0.05$ compare with positive control.

The observed antibacterial activity is primarily attributed to the presence of bioactive compounds such as flavonoids and polyphenols in tempuyung leaves. Flavonoids are known to inhibit bacterial quorum sensing, disrupt biofilm formation, and induce oxidative stress that damages bacterial cell membranes. In addition, polyphenols exhibit antibacterial activity against Streptococcus pyogenes by interfering with membrane integrity and inhibiting essential bacterial enzymes. Alkaloids may inhibit bacterial DNA replication and protein synthesis, while steroid compounds can interact with membrane lipids and alter membrane permeability, leading to bacterial cell damage [28]. In addition, the liposomal system plays a crucial role in enhancing the stability and delivery of active compounds, enabling more efficient interaction with bacterial cells and improving overall antibacterial effectiveness.

Storage stability test

The stability of TLE-LipTS was evaluated over 30 days under refrigerated (4 °C) and room temperature conditions by assessing pH, viscosity, spreadability, and antibacterial activity.

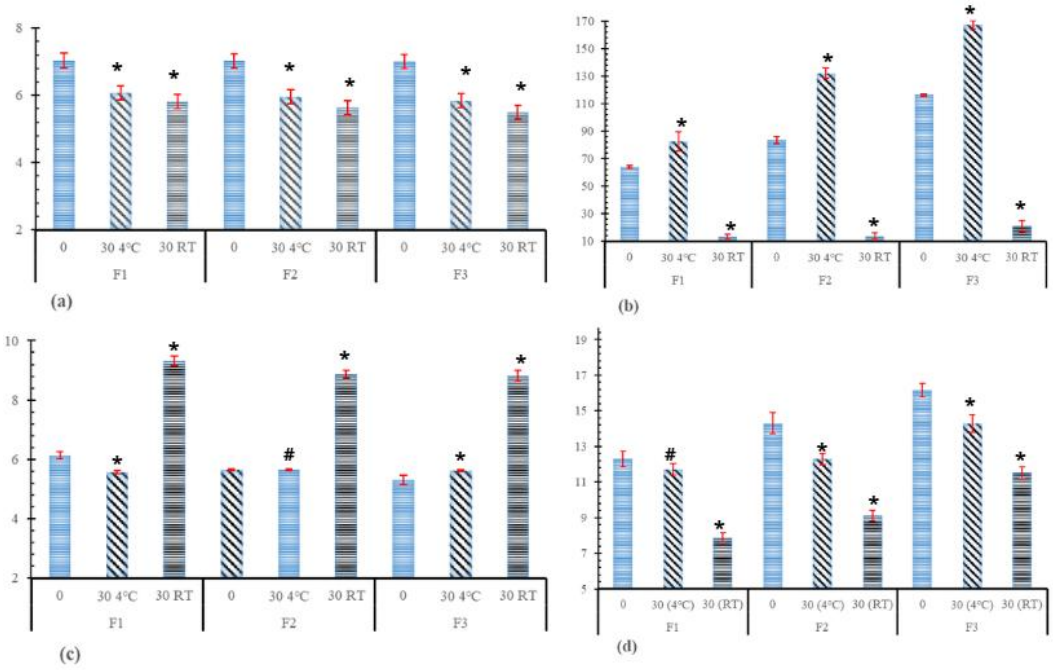


Figure 6 Stability Test Result of TLE-LipTS at Room Temperature (RT) and 4 °C for 30 Days (a) pH Measurements; (b) Viscosity Measurement (c) Spreadability (d) Antibacterial Activity. Data are presented as mean ± standard deviation (n = 3); * significance level of $p < 0.05$ and # insignificance level of $p > 0.05$ compare with F0.

The results showed that all parameters changed during storage; however, formulations stored at 4 °C exhibited smaller variations compared to those stored at room temperature. The pH values decreased from an initially near-neutral condition to slightly acidic, which may be attributed to chemical degradation of encapsulated compounds or interactions within the liposomal system during storage [39].

Changes in viscosity and spreadability also reflected the stability of the liposomal structure. The increase in viscosity at 4 °C indicates that the formulation was able to maintain its stability, whereas the significant decrease in viscosity at room temperature suggests disruption of the liposomal bilayer structure. This instability is consistent with the characteristics of aqueous liposomes, which are prone to aggregation, fusion, and leakage during storage, ultimately affecting physicochemical properties and system integrity. The increase in spreadability at higher temperatures further indicates reduced consistency due to vesicle structural breakdown [20,40].

Consistent with these changes, the stability test of antibacterial activity after 30 days of storage showed significant differences between the two temperature conditions. Prior to storage, all formulations exhibited strong antibacterial activity, with mean inhibition zones of 12.3, 14.3, and 16.17 mm, respectively. After storage at 4 °C, the antibacterial activity was relatively maintained within the strong category, with F1 at 11.7 mm, F2 at 12.3 mm, and F3 at 14.30 mm, showing only slight reductions. In contrast, storage at room temperature resulted in a more pronounced decrease, particularly in F1 and F2, which shifted from strong to moderate categories (7.9 and 9.1 mm), while F3 remained in the strong category (11.53 mm) but still showed a reduction compared to initial conditions.

These findings are consistent with Sulastri *et al*., who reported that refrigerated storage better preserves the biological activity of liposomal systems, whereas higher temperatures reduce effectiveness due to instability and formulation degradation. Temperature plays a crucial role in liposome stability as it affects bilayer integrity and permeability. Elevated temperatures increase molecular kinetic energy, enhancing phospholipid mobility, which leads to increased membrane fluidity and permeability, ultimately triggering leakage of encapsulated compounds and vesicle instability. Conversely, lower temperatures help maintain bilayer structure by reducing

molecular motion, thereby minimizing the risk of aggregation, fusion, and degradation [20].

Conclusions

This study demonstrated that the formulation of tempuyung (*Sonchus arvensis* L.) leaf extract as a liposome-based throat spray (TLE-LipTS) was able to enhance both antibacterial activity and stability. The optimized formulation (F3) exhibited the highest antibacterial activity against *Streptococcus pyogenes*, with an inhibition zone of 16.17 ± 0.38 mm, exceeding the positive control (15.3 ± 0.42 mm), indicating strong antibacterial potential. After 30 days of storage, the formulation maintained its activity under refrigerated conditions (4 °C) with only a slight decrease (14.30 mm), whereas a more pronounced reduction was observed at room temperature, confirming the influence of temperature on formulation stability.

These findings are consistent with the objective of improving the effectiveness and stability of plant extract delivery through a liposomal system. The incorporation of liposomes contributed to enhanced delivery and retention of bioactive compounds, thereby supporting increased antibacterial activity. Overall, the developed TLE-LipTS formulation shows promising potential as an alternative therapeutic approach for the treatment of pharyngitis, particularly when stored under appropriate conditions. Further studies are recommended to evaluate encapsulation efficiency (EE%), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and *in vivo* therapeutic efficacy to further confirm and optimize the antibacterial performance of the formulation.

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