



Gum Arabic-Based Microencapsulation of *Delonix regia* Extract and Its *In Vitro* Antibacterial Activity

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ABSTRACT

Delonix regia is a plant species that can be easily found in Indonesia. The bioactive compounds in *Delonix regia* leaves have potential medicinal properties but face limitations, such as instability, which may affect their biological activity. This study aimed to microencapsulate the ethanol extract from *Delonix regia* leaves using gum arabic as the coating material, employing a freeze-drying process. The effect of pH, coating material concentration, and stirring duration on encapsulation efficiency was evaluated to identify optimal conditions for microcapsule formation. The antibacterial activity of the microcapsules was assessed *in vitro* against *Escherichia coli* and *Salmonella typhimurium* using the disc diffusion method (Kirby-Bauer). The optimum conditions for microcapsule formation were achieved at pH 5, with a coating material concentration of 4% and 30 min of stirring time, resulting in an encapsulation efficiency (%EE) of 55.00%. FTIR analysis showed shifts in wave numbers and changes in peak intensity, indicating an interaction between the core material from *Delonix regia* extract and the gum arabic polymer. SEM characterisation showed the microcapsules to be mostly round but irregular, with a consistent size distribution of 3-5 µm in diameter. The microcapsules exhibited antibacterial activity at 100% concentration, producing inhibition zones of 6.06 mm against *Escherichia coli* and 6.77 mm against *S. typhimurium*.

Keywords: *Delonix regia*, gum arabic, microencapsulation, antibacterial

Introduction

Antibacterials are substances that combat pathogenic bacteria by either killing them or interfering with their metabolic activities, thereby reducing their harmful effects in the biological environment.¹ Several previous studies have demonstrated that the leaves of *Delonix regia* possess antibacterial properties.^{2,3} *Delonix regia* is a genus of flowering plants in the family Fabaceae and subfamily *Caesalpinioideae* native to Madagascar. The *Delonix regia* plant typically grows to a height of 10 to 15 meters, with some reaching a maximum of 18 meters. The leaves are light green, double-pinnate, with a somewhat fuzzy surface, and range in length from 20 to 60 cm. The plant contains several phytochemical compounds with therapeutic potential for human health.⁴ The antibacterial activity of *Delonix regia* leaves is attributed to their bioactive compounds. Among the bioactive compounds in *Delonix regia* leaves, flavonoids have been identified as key contributors to their antibacterial activity.³ In addition to flavonoids, the leaves also contain other secondary metabolites such as alkaloids, phenolics, tannins, saponins, and terpenoids, as well as amino acids and lipids, all of which are thought to play a role in its medicinal properties.²

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Flavonoids exhibit three main antibacterial mechanisms of action: inhibiting nucleic acid synthesis, disrupting cytoplasmic membrane function, and interfering with energy metabolism.⁵ However, bioactive compounds, including flavonoids, are often limited by their instability, which can reduce their biological activity. Flavonoids, a class of polyphenolic compounds with unsaturated bonds in their structure, are particularly vulnerable to environmental factors such as temperature, oxygen, water, and light.^{6,7} To address these challenges, various strategies have been developed to enhance the stability of bioactive compounds, with microencapsulation technology emerging as an effective method.

Microencapsulation is a technique of encapsulating or coating a core substance using certain polymeric materials to produce small particles termed microcapsules or microspheres. This technique is designed to improve the stability and solubility of the material while controlling the release of bioactive compounds, create solid particles with specific coatings to reduce the loss of active ingredients and shield bioactive compounds from potentially damaging environmental factors.⁹ The most common technique used in preparing microcapsules is freeze drying. The freeze-drying process involves several key stages: freezing, sublimation, desorption, and final storage to yield dried materials. One of the primary advantages of freeze-drying is that it occurs at low temperatures and in the absence of air, which helps prevent damage from oxidation and chemical degradation.¹⁰

The key factors in microcapsule preparation include the concentration of the coating material, pH, and stirring time.⁷ These factors significantly impact the encapsulation efficiency and quality of the microcapsules, making it necessary to optimise the parameters involved in the encapsulation process.¹¹ The selection of coating material is crucial, as it significantly affects the stability and quality of the encapsulated bioactive compounds.¹² Gum arabic, a biocompatible polysaccharide, is commonly used as an encapsulant due to its strong emulsifying ability, low viscosity, and non-toxic, tasteless properties.¹³

Gum arabic can form a protective layer around the core material and function as an emulsifier, preventing agglomeration by creating a thick coating.¹⁴ The emulsifying properties of gum arabic protect the core substance from damage. By serving as a protective layer, the emulsion ensures the core substance maintains its quality and stability during storage and transportation, effectively isolating it from external environmental factors.¹⁵ While the primary function of the coating material is to shield the core material from external influences, it does not directly enhance the biological activity of the encapsulated compounds.¹⁶

This research investigates using gum arabic as a coating material to protect the core compounds in *Delonix regia* extract under optimal conditions. The study explores the effects of varying coating concentrations, pH levels, and stirring times on the properties and morphology of the microcapsules. Additionally, the biological efficacy of the microcapsules, particularly their antibacterial activity, is influenced by the type of coating, including gum arabic. Understanding how gum arabic affects the antibacterial properties of *Delonix regia* extract microcapsules is essential. The primary goal of this research is to identify the optimal encapsulation conditions that maximise the protection of the core compounds of *Delonix regia* extract, as indicated by encapsulation efficiency and antibacterial activity

Materials and Methods

Materials

The leaves of *Delonix regia* were collected in August 2023 from Pukdale Village, Kupang City, East Nusa Tenggara (NTT), Indonesia, at coordinates (-10.131607108616807, 123.83898359630477). The following materials were used in the study: Acacia gum (Merck), ethanol (96% p.a.), methanol, aluminium chloride (AlCl₃), sodium acetate, sodium citrate, acetic acid, distilled water, test bacteria: *Escherichia coli* and *Salmonella typhimurium*; nutrient agar (Oxoid), sodium chloride (NaCl), blank disks (Oxoid), and ciprofloxacin antibiotic discs (Oxoid, 5 µg concentration).

Instrumentation

The instruments used include a Fourier Transform Infrared Spectrometer (Shimadzu Prestige 21), UV-visible spectrophotometer (Shimadzu Model 160A Double Beam), and Scanning Electron Microscope (TM3000 Hitachi).

Extract Preparation

Delonix regia leaves were collected during January-February 2024 from Pukdale, Kupang, Nusa Tenggara Timur, Indonesia. The plant identification was conducted by a botanist at the Department of Biology, Brawijaya University. The *Delonix regia* leaves were washed under running water, drained, and dried for 5 days in an open space, away from direct sunlight, at 25 °C. About 1 kg of dried leaves were finely ground using a blender. The powdered material (250 g) was macerated in 1 L of 96% ethanol (1:4) and allowed to stand for 72 h with intermittent stirring. The resulting mixture was filtered and concentrated using a rotary evaporator at 68 °C, and the crude extract was then kept at 4 °C for later use.

Microencapsulation Procedures

Delonix regia leaf extract (0.1 g) was dissolved in 5 mL of 96% ethanol. Subsequently, 2 g of gum arabic was dissolved in 50 mL buffer solutions at varying pH levels (3, 4, 5, and 6). The *Delonix regia* leaf extract solution was mixed with the gum arabic solution, and 150 mL of distilled water was added. The mixture was stirred using a magnetic stirrer for 30 minutes until well mixed. Finally, the prepared sample was freeze-dried until a dry powder was obtained. Additionally, microcapsules were fabricated following the same protocol to examine the effects of varying gum arabic concentrations of 2%, 4%, 6%, and 8% (w/v). The starting pH that achieved the best encapsulation efficiency was utilised, while other parameters remained constant. Finally, microcapsules were prepared by examining the impact of varying stirring durations using the same procedure, with stirring times of 15, 30, 45, and 60 min. The preparation was performed using pH buffer solution and gum arabic concentration at optimum conditions.

The optimum condition for microcapsules for each parameter was determined by calculating the percentage value of encapsulation efficiency (%EE). The highest %EE value represents the optimum condition of the microcapsules, which is determined from the total flavonoid content in *Delonix regia* leaf extract and the microcapsules using the following equation.¹⁷

Encapsulation

$$\text{Efficiency (\%)} = \frac{\text{Total flavonoid content in microcapsules}}{\text{Total flavonoid content in the extract}} \times 100\% \quad (1)$$

Determination of Total Flavonoid Content

Total flavonoid content (TFC) was assessed using a colourimetric method with AlCl₃ and quercetin as the standard drug. The standard curve was constructed using quercetin (QE) solutions at different concentrations ranging from 5 to 20 µg/mL. The sample (*D regia* leaf extract and microcapsules) was dissolved in 5 mL of methanol and incubated for 45 min at 40 °C. After incubation, the solution was centrifuged for 10 min. After that, 0.6 mL of the generated solution was combined with 0.6 mL of 2% aluminium chloride, and the combination was then let to stand for 60 min at room temperature. The absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength of quercetin (420 nm). The total flavonoid content of the extracts and microcapsules was determined using a plot of the quercetin standard curve ($y = 0.0392x$, $R^2 = 0.964$) and reported as milligrams of quercetin equivalent per gram of the extract (mgQE/g).¹⁸ The absorbance value was used to calculate the total flavonoid content in the sample according to the following formula:¹⁵

$$\text{Total Flavonoid Content (TFC)} = \frac{\text{Sample concentration} \left(\frac{\text{mg}}{\text{mL}} \right) \times \text{Volume (mL)}}{\text{Sample weight (g)}} \quad (2)$$

Antibacterial Activity Assay

The disc diffusion method, or the Kirby-Bauer technique, was used to test for antibacterial properties.² *Escherichia coli* and *S. typhimurium* were cultured in nutrient agar (NA) media. Subsequently, bacterial suspensions were prepared using a 0.9% NaCl solution, and optical density (OD) values were measured with a UV-Vis spectrophotometer at a wavelength of 600 nm, with a cell density of 0.6, equivalent to 10⁶ CFU/mL bacteria. The bacterial suspension was then collected using a sterile cotton bud and evenly applied to the surface of the NA media in a Petri dish. Sterile paper discs were immersed in the microcapsule samples for 30 min at various concentrations: 25%, 50%, 75%, and 100% (w/v). After 30 min, the discs were removed using sterile tweezers and placed onto the surface of the media inoculated with bacterial suspensions. The plates were incubated for 24 hours at 37 °C. Ciprofloxacin discs were used as the positive control, while 10% ethanol solvent was the negative control (the experiment was conducted in triplicate). The clear zone that developed after the incubation time was measured using a calliper and reported in millimetres (mm).

Characterisation of Microcapsules

FTIR-ATR characterisation was used to identify the functional groups in *Delonix regia* leaf ethanol extract and microcapsules under optimum conditions. The sample was placed on the FTIR machine's plate to cover the crystal, and the spectrum was then recorded using a computer application connected to the FTIR instrument.

SEM characterisation was used to analyse the morphology of *Delonix regia* leaf extracts and microcapsules at magnifications ranging from 6000 to 15,000x.

Data Analysis

The data analysis was conducted using IBM SPSS Statistics software version 29.0. The microencapsulation process in optimum conditions, including pH concentration of encapsulating material and stirring time, was analysed through normality and homogeneity tests. A one-way ANOVA test was conducted with a 95% confidence level ($\alpha = 0.05$). Tukey's post hoc test assessed significant differences among the treatments, with a $p < 0.05$ considered statistically significant.¹⁹

Results and Discussion

Microencapsulation of *Delonix regia* Leaves Extracts

The total flavonoid content was determined using a colourimetric method, with quercetin as the standard for calibration curve preparation.¹⁶ Flavonoids are polyphenolic compounds known for their antibacterial properties through various mechanisms. Based on the results, the total flavonoid content (TFC) in *Delonix regia* leaf extract was 9.86 ± 0.03 mg QE/g. This indicates that each gram of extract contains a flavonoid equivalent to 9.86 mg of quercetin.

Microcapsules of *Delonix regia* leaf extract were produced using the freeze-drying technique. The microencapsulation process is influenced by three key parameters: pH, gum arabic concentration, and stirring time. Table 1 presents the percentage of encapsulation efficiency for *Delonix regia* leaf extract microcapsules produced by varying these parameters. The optimal conditions for microencapsulation were determined to be a pH of 5 in acetate buffer, a coating material concentration of 4% (b/v), and a stirring time of 30 min, which resulted in the highest encapsulation efficiency of 55.00%. The results indicate that pH significantly affects encapsulation efficiency. Microcapsules prepared at pH 5 exhibited the highest efficiency, while those prepared at pH 3 showed the lowest efficiency. This can be attributed to the high concentration of H⁺ ions in acidic solutions, which reduces carboxyl groups (-COOH) ionisation on polysaccharides. As a result, the electrostatic repulsion between carboxyl groups decreases, leading to lower viscosity in the polysaccharide solution. Consequently, the microcapsule matrix becomes weaker, preventing effective encapsulation of the active compounds from *Delonix regia* leaf extract and reducing encapsulation efficiency.¹⁹

From pH 4 to pH 5, the encapsulation efficiency increases, consistent with previous research¹⁹, which confirms that as pH rises, the dissociation of acidic functional groups on carbohydrates enhances electrostatic repulsion, which expands the molecular dimensions and affects the viscosity of the polysaccharides, optimising their interactions. Gum arabic maintains stable viscosity at pH 5 and a moderate acidity around pH 4.7 to 5.4. Gum arabic is a macromolecular acid that contains minerals such as magnesium (Mg), calcium (Ca),

potassium (K), iron (Fe), and sodium (Na) from acidic polysaccharides; this acidity is attributed to the presence of uronic acid in its structure.^{20,21} Hence, microcapsules prepared at pH 5 using acetic acid exhibited the highest encapsulation efficiency. In contrast, at pH 6, encapsulation efficiency decreased. Previous studies have shown that at slightly acidic pH levels, the hydrogen bond structure in polysaccharide molecules weakens or becomes unstable, reducing their ability to interact efficiently with solvents and decreasing electrostatic repulsion, resulting in lower viscosity¹⁸ and a weakened microcapsule matrix, making it less effective in encapsulating and retaining the active ingredients of the *Delonix regia* leaf extract, ultimately lowering encapsulation efficiency.

In this study, the optimum microencapsulation condition was achieved with a gum arabic concentration of 4% (w/v). At a concentration of 2%, the encapsulation efficiency was lower. At 6% and 8% concentrations, the encapsulation efficiency decreased again. This reduction is attributed to the fact that a higher concentration of gum arabic results in a denser matrix structure and smaller pore sizes, which limits the number of active ingredients that can enter or be successfully encapsulated, thereby decreasing the encapsulation efficiency. Conversely, a lower concentration of the polymeric material creates larger pore sizes, facilitating the entry of active ingredients and allowing a greater amount to be encapsulated, which increases the encapsulation efficiency.²²

Other factors, such as pH, coating material concentration, and stirring duration, significantly impact the shape and size of the resulting microcapsules.⁹ As shown in Table 1, the optimum stirring time for microcapsules is 30 minutes because it produces the highest encapsulation efficiency compared to stirring times of 15, 45, and 60 minutes. Excessively fast stirring leads to particle aggregates forming, resulting in larger and less smooth microcapsules. Larger microcapsules absorb fewer active ingredients into the matrix, leading to a lower encapsulation efficiency. In contrast, prolonged stirring times can produce microcapsules with brittle characteristics, making them prone to breakage. This brittleness causes the microcapsules to rupture prematurely, resulting in the early release of the active ingredients.^{15,18}

Table 1: Encapsulation efficiency of microcapsules of *Delonix regia* leaf extract

pH*	%EE**	Concentration of gum arabic (% w/v)*	%EE**	Stirring time (min)*	%EE**
3	32.67 ± 0.12 ^b	2	48.19 ± 0.14 ^c	15	46.93 ± 0.13 ^c
4	41.97 ± 0.33 ^c	4	55.19 ± 0.17 ^d	30	55.00 ± 0.17 ^d
5	52.58 ± 0.38 ^d	6	38.55 ± 0.09 ^b	45	45.03 ± 0.10 ^b
6	26.55 ± 0.14 ^a	8	36.77 ± 0.14 ^a	60	40.93 ± 0.14 ^a

*Samples of microcapsules with variations in pH, concentration of gum Arabic and stirring times.

**Distinct notations signify significant differences between conditions, as determined by the one-way ANOVA test with a confidence level of $\alpha = 0.05$

Characterization of Microcapsules

FTIR characterisation was employed to identify the functional groups of the microcapsules prepared under optimal conditions. The spectral results were then compared with the FTIR spectra of the *Delonix regia* leaf extract and the coating material (gum arabic). This comparison was conducted to assess whether the active ingredients of the extract were effectively encapsulated within the gum arabic matrix. The FTIR characterisation results in the absorption region of 4000-500 cm⁻¹ are shown in Figure 1, with a detailed analysis presented in Table 2. The success of the encapsulation process is indicated by the results of the IR spectra, which show the presence of specific peaks from *Delonix regia* leaf extract that appear in the IR spectra of the microcapsules. These peaks exhibit shifts in wavenumbers and changes in intensity, indicating interactions between the core material and the gum arabic coating. The first peak exhibits a shift in wavenumber and a change in intensity, from 3305.96 cm⁻¹ to 3408.65 cm⁻¹ in the microcapsules, while the second peak shows a shift from 3274.58 cm⁻¹ in gum arabic to 3277.43 cm⁻¹ in

the microcapsules. This shift is attributed to the interaction between the active compounds in the extract and gum arabic via hydrogen bonding, causing the hydroxyl group (O-H) to shift to a higher wavenumber. The absorption band at 1544.59 cm⁻¹ corresponds to the aromatic C=C functional group of polyphenols, a characteristic of the flavonoid structure in *Delonix regia* leaf extract.² Additionally, the microcapsules exhibit specific peaks at 1637.29 cm⁻¹ and 1406.24 cm⁻¹, corresponding to the asymmetric and symmetric stretching of the carboxylate group (COO⁻), respectively, which are characteristic of the gum arabic structure.^{23,24}

Scanning electron microscopy (SEM) analysis was conducted to examine the surface morphology and shape of the microcapsules. Figure 2 shows the different SEM results of *Delonix regia* leaf microcapsules and extracts at 6,000x magnification. The microcapsules exhibit a generally round but irregular shape with a relatively uniform size, approximately 3-5 μm in diameter. The irregular or uneven surface of the microcapsules may be due to the drying technique used during

the microencapsulation process. Previous research²⁵ revealed that freeze-drying produces microcapsules with irregular surfaces, such as dents or concavities. Freeze-drying, a method that uses low

temperatures, causes ice crystals to form, creating cavities in the microcapsules, resulting in an irregular surface structure.²²

Table 2: Interpretation of the FTIR spectra from Figure 1

Peak number	Microcapsules of <i>Delonix regia</i> leaf extract	Gum arabic	<i>Delonix regia</i> leaf extract
1	3408.65 cm ⁻¹ for O-H hydroxyl	3274.58 cm ⁻¹ for O-H hydroxyl	3305.96 cm ⁻¹ for O-H hydroxyl
2	3277.43 cm ⁻¹ for O-H hydroxyl	2935.14 cm ⁻¹ for C-H alkane	2922.31 cm ⁻¹ for C-H alkane
3	3164.76 cm ⁻¹ for O-H hydroxyl	1635.66 cm ⁻¹ for COO- asymmetric carboxylate	2852.42 cm ⁻¹ for C-H alkane
4	1691.49 cm ⁻¹ for C=O carbonyl	1421.93 cm ⁻¹ for COO- symmetric carboxylate	1735.70 cm ⁻¹ for C=O carbonyl
5	1637.29 cm ⁻¹ for COO- asymmetric carboxylate	1019.74 cm ⁻¹ for C-O-C ether	1608.77 cm ⁻¹ for C=C aromatic
6	1544.59 cm ⁻¹ for C=C aromatic		1516.06 cm ⁻¹ for C=C aromatic
7	1406.24 cm ⁻¹ for COO- symmetric carboxylate		1447.60 cm ⁻¹ for C=C aromatic
8	1018.32 cm ⁻¹ for C-O-C ether		1042.56 cm ⁻¹ for C-O-C ether
9	797.25 cm ⁻¹ for C-H aromatic		817.22 cm ⁻¹ for C-H aromatic
10	638.94 cm ⁻¹ for C-Cl alkyl halide		

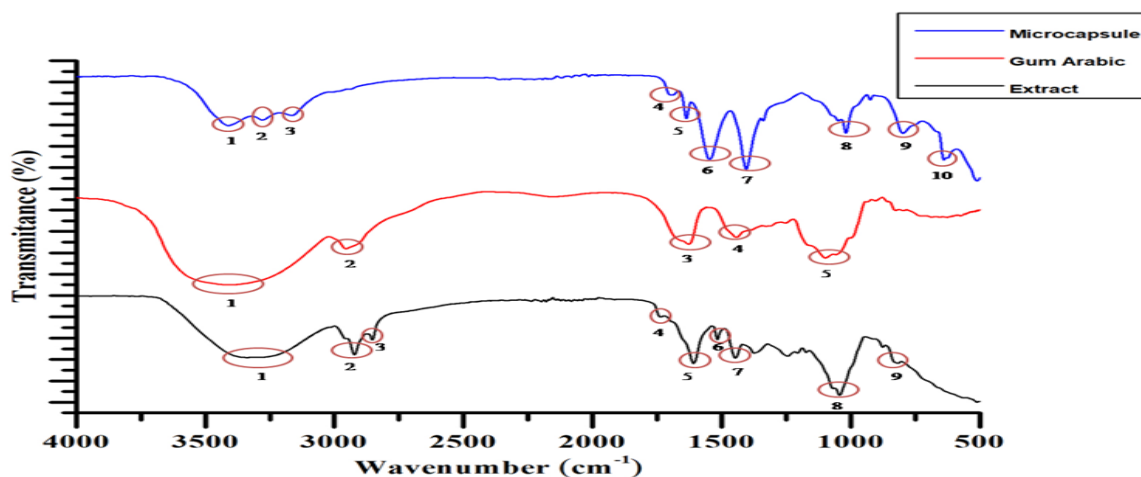
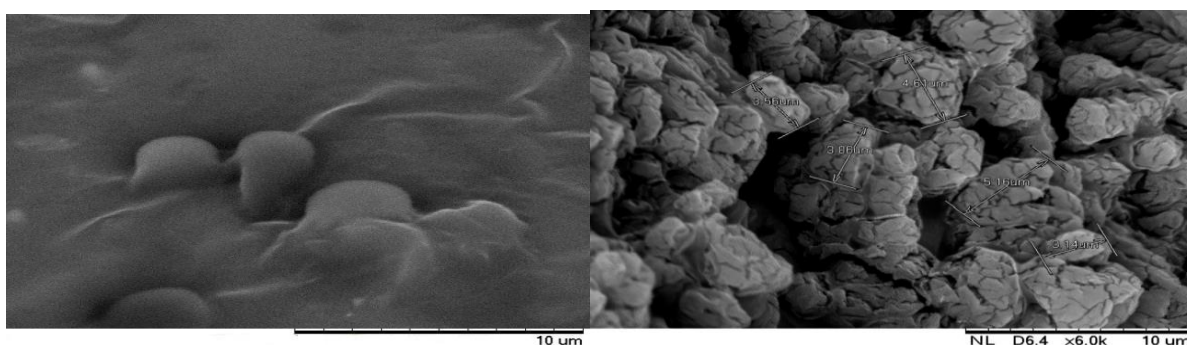


Figure 1: FTIR spectra of *Delonix regia* leaf extract, gum arabic, and *Delonix regia* leaf extract microcapsules prepared at pH 5, 4% (b/v) gum arabic, and 30 min stirring time.



(a) (b)
Figure 2: SEM images of the: (a) *Delonix regia* leaf extract; (b) Microcapsules of *Delonix regia* leaf extract prepared under the optimum conditions of pH 5, 4% (b/v) gum arabic, and 30 minutes stirring time. Magnification: 6,000 \times .

Antibacterial Activity Assay

In the antibacterial study, flavonoid compounds present in *Delonix regia* leaf extract demonstrated antibacterial properties. This study investigated the antibacterial activity of *Delonix regia* leaf extract encapsulated with gum arabic as the coating material. Microcapsules were prepared under optimal conditions at four different concentrations: 25%, 50%, 75%, and 100% (w/v). This variation was designed to identify the most effective concentration for inhibiting the growth of *Escherichia coli* and *Salmonella typhimurium*. The results of the antibacterial activity test of the *Delonix regia* leaf extracts are shown in Figure 3, while Figure 4 shows results of antibacterial activity test of microcapsules of the *Delonix regia*. The average diameters of the inhibition zones are presented in Table 3. Table 3 indicates that the inhibition zone diameters vary and increase with rising microcapsule concentrations, with the 100% (w/v) concentration producing the largest zones: 6.06 mm against *Escherichia coli* and 6.77 mm against *S. typhimurium*. According to the classification by David & Stout², the 100% concentration falls into the category of moderate inhibition, while the 25%, 50%, and 75% concentrations are classified as weak inhibition. It is important to note that the primary purpose of microencapsulation is not to enhance biological activity but to protect bioactive compounds from adverse environmental conditions (e.g., enzymes, pH, temperature, light, oxygen, and moisture) and to control the release of bioactive compounds.¹⁷ Controlled or gradual release aims to protect the bioactive compounds from environmental stressors that can reduce their stability and effectiveness. It also ensures that the active ingredients are released at the right time and location to produce

the desired therapeutic effect. Flavonoids (polyphenolic compounds) contribute to the antibacterial activity of *Delonix regia* leaf microcapsules. These compounds can inhibit bacterial growth through several mechanisms, including the inhibition of nucleic acid synthesis (DNA and RNA), disruption of cytoplasmic membrane function, inhibition of energy metabolism, prevention of bacterial attachment and biofilm formation, and interference with porin function in the cell membrane. Additionally, flavonoids can reduce bacterial pathogenicity (the ability to cause disease) and alter bacterial cell membrane permeability, further contributing to their antibacterial effect.²⁶ Previous studies revealed that hydroxyl groups on the aromatic rings of flavonoid structures play a crucial role in their antibacterial activity. The flavonoid structure (C6-C3-C6) consists of three rings—two phenyl rings and one heterocyclic ring (C)—with hydroxyl groups on the A ring, particularly at the C-7 position, crucial for antibacterial activity; additional hydroxyl groups at the C-5 and C-6 positions can enhance this effect.²⁷ The positive control (ciprofloxacin) exhibits the largest inhibition zone diameter, which was very strong against *Escherichia coli* and *S. typhimurium*. Ciprofloxacin is a fluoroquinolone-class antibiotic with a broad spectrum of activity commonly used to treat infections caused by Gram-positive and Gram-negative bacteria, including those affecting the urinary, respiratory, skin, and gastrointestinal tracts.^{28,29} In contrast, the negative control (10% ethanol) showed no inhibition zones around the disc for either test bacteria, indicating that 10% ethanol, used as a solvent, lacks antibacterial properties.

Table 3: Average inhibition zone diameter of microcapsules of *Delonix regia* leaf extract

Bacteria	Mean Inhibition Zone Diameter (mm) \pm SD					
	25%	50%	75%	100%	Control +	Control -
<i>Escherichia coli</i>	1.96 \pm 0.31	3.31 \pm 0.41	4.97 \pm 0.26	6.06 \pm 0.05	27.77 \pm 0.13	0
<i>Salmonella typhimurium</i>	1.46 \pm 0.45	3.36 \pm 0.10	4.35 \pm 0.08	6.77 \pm 0.16	24.22 \pm 0.48	0

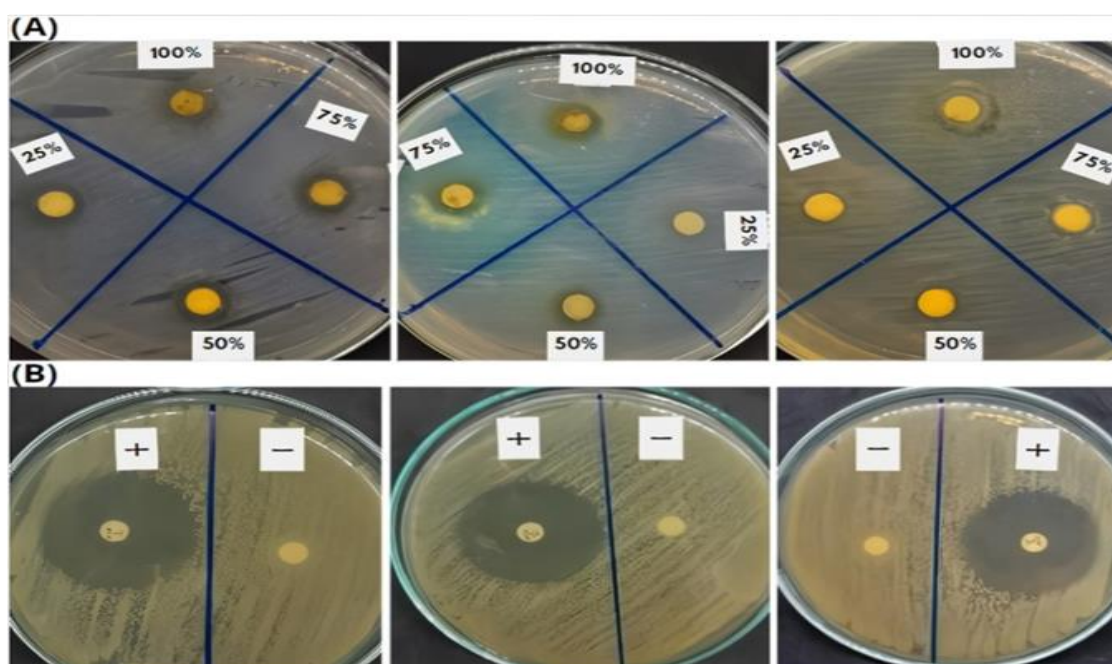


Figure 3: Antibacterial activity test of microcapsules of *Delonix regia* leaf extract against *Escherichia coli*: (A) microcapsules at concentrations of 25%, 50%, 75%, and 100% (replicates 1, 2, and 3); (B) positive control (ciprofloxacin) and negative control (10% ethanol) (replicates 1, 2, and 3).

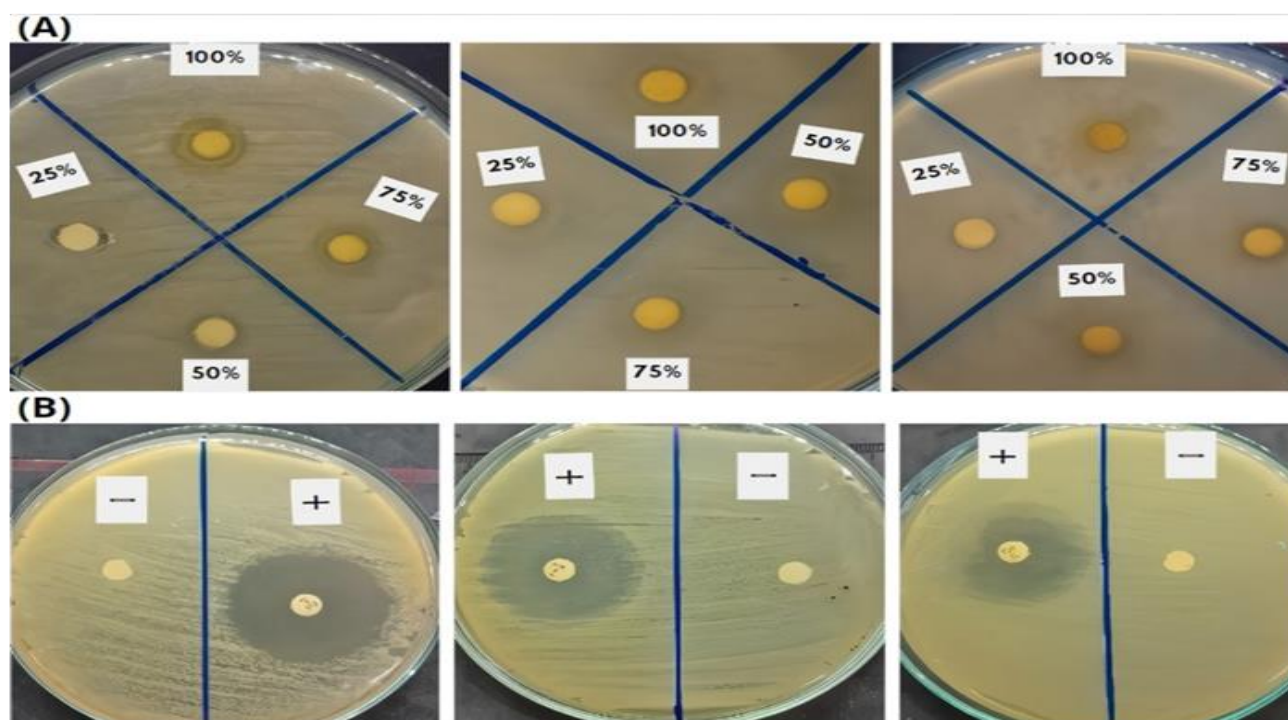


Figure 4: Antibacterial activity test of microcapsules of *Delonix regia* leaf extract against *Salmonella typhimurium*: (A) Microcapsules at concentrations of 25%, 50%, 75%, and 100% (replicates 1, 2, and 3); (B) Positive control (ciprofloxacin) and negative control (10% ethanol) (replicates 1, 2, and 3).

Conclusion

This research successfully demonstrated the microencapsulation of *Delonix regia* leaf extract using gum arabic as the encapsulation material through the freeze-drying technique. The optimum microencapsulation conditions were achieved with an acetate buffer at pH 5, a 4% (w/v) gum arabic concentration, and a 30-minute stirring time, resulting in an encapsulation efficiency of 55.00%. This finding suggests that gum arabic can effectively protect the flavonoid compounds in *Delonix regia* leaves. The success of the encapsulation process was further confirmed through characterisation using FTIR. *In vitro* antibacterial activity analysis showed that microcapsules at the highest concentration effectively inhibited the growth of test bacteria. These results indicate that *Delonix regia* microcapsules can serve as potential antibacterial agents. Future research should focus on *in vivo* testing of the antibacterial activity of *Delonix regia* leaf microcapsules and additional *in vitro* assays, such as antioxidant activity and alpha-amylase inhibition assays.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declarations

The authors declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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