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Encapsulation of Lamtoro Leaf Extract (*Leucaena Leucocephala (Lam.) De Wit*) using Chitosan and Crosslink Agent Sodium Tripolyphosphate

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Abstract:- Rice is a vital global staple, faces challenges in production due to factors such as diminishing fertile land, natural disasters, and persistent pest threats. Traditional pesticides raise environmental concerns, prompting the exploration of eco-friendly alternatives. Lamtoro leaf extract, known for its pesticidal properties, poses challenges in solubility and vulnerability. Encapsulation with chitosan, a biocompatible polysaccharide, emerges as a solution. This study addresses the research gap by encapsulating lamtoro leaf extract with chitosan crosslinked with sodium tripolyphosphate and explores its application in rice seed germination. The encapsulation process is comprehensively investigated, considering lamtoro leaf extract concentrations, crosslink agent, and Tween 80 effects on encapsulation efficiency, particle size, and microcapsule yield. The study further assesses the impact on rice seed germination, aiming to contribute to sustainable agricultural practices.

Keywords:- *Lamtoro leaf extract; Chitosan; Encapsulation; Pesticides; Seed germination; Microcapsules*.

I. INTRODUCTION

Rice, a staple food for nearly half of the global population, holds critical significance, particularly in Asia where 90% of the world's rice is cultivated and consumed [1] [2]. The importance of rice production is underscored by the fact that, in 2019, a staggering 756 million tons of rice were produced globally [3]. Notably, Indonesia stands as the third-largest producer of rice, contributing 54.6 million tons to the global production in 2019 [3]. As the world's population continues to grow, the demand for rice production must parallel this expansion. However, rice production faces multifaceted challenges, including diminishing fertile land due to urbanization and industrialization, recurring natural disasters like droughts and floods, and the persistent threats of pests and diseases [4] [5].

To tackle these challenges, enhancing seed quality through pre-sowing treatments is a pivotal approach. Commonly, pesticides are employed for their ability to safeguard seeds against pathogenic fungi and bolster germination rates [6]. However, the extensive use of chemical pesticides raises environmental concerns, leading to issues such as hazardous residues, pest resistance, and export restrictions [7].

In light of these challenges, botanical pesticides present a promising alternative, advocating for sustainable agricultural practices. Plant-derived pesticides, with their bioactive compounds like phenolic compounds, alkaloids, and terpenoids, offer an environmentally friendly solution [8]. Lamtoro (Leucaena leucocephala (Lam.) de Wit) is one such plant with potential pesticidal properties, traditionally used as a cover crop to control erosion and enhance soil fertility [9]. Lamtoro leaf extract has shown effectiveness against various pests, making it a viable option for pest management in crops like oil palm [9].

However, the application of lamtoro leaf extract faces challenges related to the inherent characteristics of its bioactive compounds. These compounds, including mimosine, saponins, and flavonoids, exhibit low solubility in water and vulnerability to environmental factors such as oxygen, light, heat, and humidity [10]. To address these challenges, encapsulation emerges as an effective strategy to protect these bioactive compounds and minerals under adverse environmental conditions.

Encapsulation involves coating the active ingredients with a protective material, and chitosan, derived from chitin, stands out as a promising encapsulating agent. Chitosan, a linear polysaccharide, possesses biocompatibility, biodegradability, and antimicrobial properties, making it an attractive choice for encapsulation [6] [11]. However, chitosan in its gel form can be brittle, prompting the addition of crosslink agents such as sodium tripolyphosphate to enhance its rheological properties [12].

While previous studies have explored the encapsulation of various herbal extracts using chitosan and crosslink agents, there is a research gap regarding the encapsulation of lamtoro leaf extract using chitosan crosslinked with sodium tripolyphosphate. This study aims to address this gap and contribute to the existing knowledge in the field. Furthermore, the research introduces innovation by applying the encapsulation product to rice seed germination. This encapsulation product could offer an eco-friendly alternative to chemical pesticides in rice cultivation, promoting sustainable and environmentally conscious agricultural practices. This research aims to comprehensively explore the encapsulation process of lamtoro leaf extract, focusing on understanding the influence of varying concentrations of lamtoro leaf extract on encapsulation efficiency, particle size, and microcapsule yield. Additionally, the study seeks to investigate the

impact of different concentrations of crosslink agent and Tween 80 on these encapsulation parameters. Furthermore, the research aims to assess the effect of encapsulating lamtoro leaf extract in chitosan/sodium tripolyphosphate on rice seed germination. This multifaceted approach aims to contribute valuable insights into optimizing the encapsulation process for lamtoro leaf extract, with the ultimate goal of promoting sustainable agricultural practices, particularly in the context of rice cultivation.

II. MATERIALS AND METHODS

A. Materials

Lamtoro leaf *(Leucaena leucocephala (Lam.) de Wit)*, Chitosan (degree of deacetylation 96.1%, PT. Pandu Biopolimer), Sodium Tripolyphosphate / Na-TPP (Sigma), Technical ethanol (Merck), Acetic acid / CH3COOH (Merck), Ultra-Pure Water (BPMPT), NaOH (Merck), Tween 80 (Merck), Sertani Hybrid Rice Seeds (Toko Tirta Nirmala).

B. Research Procedure

➢ *Extraction of Lamtoro Leaf*

Lamtoro leaves were gathered from wild-growing plants around Curug Village, Cimanggis Subdistrict, Depok City. The leaves were washed with flowing water, drained, and separated from the stems. Subsequently, the leaves were dried in an oven at 80 ℃ for 3 hours to reduce moisture content. Once dried, the leaves were ground using a grinder [13]. A total of 50 grams of lamtoro leaf powder (Leucaena leucocephala (Lam.) de Wit) was immersed in 300 ml of 70% ethanol, left for 6 hours with periodic shaking. The mixture was then left undisturbed for 18 hours at room temperature. The mixture was filtered, and the filtrate was collected. The residue underwent maceration again using the same procedure with 2 repetitions [14]. The entire maceration filtrate was collected and concentrated using a rotary evaporator at 60 ℃ [15].

➢ *Encapsulation of Lamtoro Leaf Extract Using Chitosan/Sodium Tripolyphosphate*

The encapsulation process follows the study conducted by Mondéjar-López et al. [6] with modifications. A total of 100 ml of chitosan solution (1% w/v), with a pH adjusted to 4–5, was prepared. Subsequently, 5 ml of the lamtoro leaf extract solution (in ethanol) and Tween 80 (each with concentrations according to the variable) were added while stirring at room temperature for 30 minutes until homogeneous. Then, a 25 ml solution of sodium tripolyphosphate was added drop by drop, following the variable, while stirring continuously. After the addition was complete, stirring continued for 45 minutes to perfect the microcapsule formation process. The suspension was then centrifuged at 6000 rpm for 15 minutes. The supernatant was separated, and the precipitate was washed with ultra-pure water twice, followed by drying in an oven at 40 ℃ for 6 hours.

C. Analysis

➢ *Phytochemicals screening*

The identification of lamtoro plant samples was carried out through determination tests at the Herbarium Depokensis, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok.

Phytochemical screening was conducted to determine the content of active ingredients in lamtoro leaves. In this study, three types of screening were performed: tube method, GCMS-MS method, and HPLC method.

➢ *Analysis of Total Flavonoid Content in Lamtoro Leaf Extract*

The total flavonoid content in the lamtoro leaf extract was calculated as mg Quercetin Equivalent per gram of the sample. The method used was the colorimetric method using a UV-Vis spectrophotometer (Shimadzu). A 1 ml sample of lamtoro leaf extract was dissolved in ethanol, and 1 ml was pipetted. The sample solution was then mixed with 1 ml of 2% AlCl3 and 1 ml of 1 M Potassium Acetate. After incubating the solution for 30 minutes, the absorbance was measured at a wavelength of 438 nm. The absorbance of the sample was then entered into the linear regression equation of the quercetin standard series (0.5, 1, 5, 10, and 20 mg/L) to obtain the flavonoid concentration (mg/L). The Flavonoid Content is expressed as mg Quercetin Equivalent per Gram of the sample.

➢ *Encapsulation Efficiency (%)*

Encapsulation efficiency measurement is calculated based on the amount of flavonoid contained in the microcapsules using the aluminum chloride colorimetric method. A 0.5 ml aliquot of the supernatant is added to 2.8 ml of ultra-pure water, followed by ethanol, 10% AlCl3, and potassium acetate (1 mol/L) in amounts of 1.5 ml, 0.1 ml, and 0.1 ml, respectively. The mixture is incubated for 30 minutes, and then its absorbance is measured at the optimum wavelength. A quercetin standard solution at concentrations ranging from 0.5 to 20 µg/mL is used as a reference [16]. The encapsulation efficiency is calculated following the formula below [11]:

$$EE\ (\%) = \frac{X0 - Xt}{X0}\ x\ 100\ \%$$

Where:
EE=Encapsulation efficiency (%)
X0=initial flavonoid level (mg)
Xt = escaped flavonoid content (mg)

➢ *Microcapsule Yield*

Microcapsules obtained after the drying process are weighed to determine their weight. The weight of the microcapsules is then compared to the total weight of the microcapsule-forming materials and expressed as yield. The yield is calculated based on the dry weight, using the formula below:

$$Yield\ (\%) = \frac{Weight\ of\ Microcapsules\ (g)}{Weight\ of\ Microcapsule - Forming\ Materials\ (g)}\ x\ 100\ \%$$

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➢ *Particle Size*

The particle size of chitosan-lamtoro leaf extract microcapsules is analyzed using the Laser Particle Size Analyzer LLPA-C10.

➢ *Scanning Electron Microscopy (SEM)*

The surface morphology of particles is analyzed using Scanning Electron Microscopy (SEM) with a JEOL JJM 7000 series instrument.

➢ *Fourier Transform Infrared (FTIR)*

Functional groups of chitosan, chitosan nanoparticles, lamtoro leaf extract, and chitosan-lamtoro leaf extract microcapsules are analyzed in the wavelength range of 4000 to 400 cm^{-1} using a Shimadzu Fourier Transform Infrared instrument.

➢ *Active Ingredient Release Study*

The release study of the active ingredient is conducted to assess the ability of the encapsulating material to control the release of the active ingredient over a specified time interval. The method follows the research by Yousefi et al. [16] with modifications. Twenty milligrams of microcapsules are weighed and dispersed in 30 ml of pH 7 buffer solution (Phosphate Buffer Saline), pH 4.3, and pH 1.3 (0.1 N HCl). The mixture is stirred at 100 rpm at room temperature for 24 hours. At specific time intervals, 1 ml of the solution is taken, and 1 ml of 2% AlCl3 and 1 ml of 1 M Potassium Acetate are added. The absorbance of the sample is measured using a UV-Vis Spectrophotometer (Shimadzu) at a wavelength of 439 nm.

➢ *In Vitro Germination Analysis of Rice Seeds*

Inbrida Sertani rice seeds are tested for germination percentage according to ISTA 2023.

III. RESULTS AND DISCUSSIONS

A. *Extraction of Lamtoro Leaf*

The plants used in this study have undergone identification at the Herbarium Depokensis, Department of Biology, Universitas Indonesia, and have been confirmed as Lamtoro plants with the Latin name *Leucaena leucocephala (Lam) de Wit*, which belongs to the *Fabaceae* family. Fresh leaves were collected from wild-growing Lamtoro plants in the yards around Depok City, West Java. The extraction of Lamtoro leaf was obtained through the maceration method. The yield produced from the maceration of 50 grams of the plant material was 3.1999 grams (ethanol extract of Lamtoro leaves), equivalent to 6.4%.

The maceration method was chosen for its simplicity and avoidance of high temperatures, minimizing the degradation of active compounds, especially heat-sensitive flavonoids. The maceration process relies on the polarity of the solvent to extract various active compounds present in Lamtoro leaves. The choice of 70% ethanol as the solvent is based on research [14], where this solvent contains a higher water content, and the -OH group in water is a highly polar compound. This characteristic allows 70% ethanol to extract both polar and non-polar compounds such as flavonoids, alkaloids, tannins, steroids, and polyphenols from Lamtoro leaves [17]. Additionally, ethanol has the advantage of a low boiling point, non-toxicity, safety, and it does not damage the active components in the leaves, as evidenced by the absence of color changes during the maceration process.

➢ *Phytochemical Screening by tube method*

Phytochemical screening of Lamtoro leaf extract was qualitatively conducted to determine the presence of secondary metabolite compounds.

Phytochemical screening with the tube method is a simple screening method that does not involve specific instrumentation. Positive results are identified by the formation of precipitates or changes in the color of the solution, as observed in Table 1.

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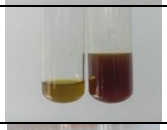
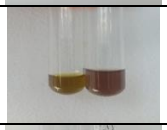
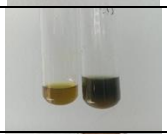
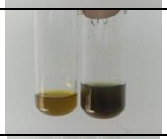
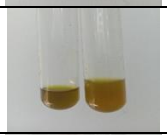
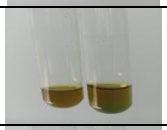
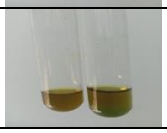
Table 1: Results of phytochemical screening of lamtoro leaf extract using tube test

Compounds	Observation result	Description	Photo
Alkaloids (Mayer method)	Precipitate formed	+	
Alkaloids (Wagner method)	A brown precipitate was formed	+	
Flavonoids	A brick red color was formed	+	
Tannins	Precipitate formed	+	
Polyphenols	A darker, blackish green color was formed	+	
Saphonin	No persistent foam was formed	-	
Steroid	The color changed to green	+	
Terpenoid	A brownish ring was formed, and no purple or orange color was observed	-	

Based on the tube test results, it was found that Lamtoro leaf extract positively contains alkaloids, flavonoids, tannins, polyphenols, and steroids. These findings align with a study conducted by Ermi Abriyani and Neneng Nurfalah in 2019. Conversely, saponins and terpenoids yielded negative results. In the saponin test, no stable foam was formed, and in the terpenoid test, there was no formation of purple or orange color. The alkaloid test on Lamtoro leaf extract produced positive results with both Wagner and Mayer reagents. The addition of Mayer reagent resulted in a precipitate believed to be a complex of potassium-alkaloid formed through the reaction of nitrogen ions in alkaloids with potassium ions from potassium tetraiodomercurate (III). Meanwhile, the brown precipitate formed in the Wagner test is believed to be potassium-alkaloid complex (resulting from the reaction between alkaloid nitrogen and potassium ions), and the brown color is produced by I3- ions (resulting from the reaction between iodine and I- ions in Wagner reagent preparation). Alkaloids, known to have antibacterial, insecticidal, and antimicrobial properties, contribute significantly to the medicinal potential of Lamtoro leaves.

In the flavonoid test, the addition of Mg powder and concentrated HCl aimed to reduce flavonoid compounds, resulting in a red, yellow, or orange-colored complex. The red color obtained in this study indicates that Lamtoro leaf extract is positive for flavonoids. Flavonoids such as quercetin, quercetin 3-O-rhamnoside, and D-Onanitol are known to exhibit antifeedant activity against Spodoptera litera. They also function as antimicrobial, antivirus, anti-inflammatory, antioxidant, and analgesic agents.

Lamtoro leaf extract, when treated with Lieberman-Burchard reagent, exhibited a color change to green, indicating oxidation of steroid compounds through the formation of conjugated double bonds. Steroids are known for their insecticidal activity. The application of a 5% FeCl3 solution resulted in a dark greenish color due to the presence of polyphenolic compounds, which are acidic, easily oxidized, and form polymers causing dark color. Polyphenols and tannins, often found together, contribute to the insecticidal, antimicrobial, astringent, antibacterial, antioxidant, and antimalarial activities of the plant.

➢ *Screening of Quercetine by HPLC*

The screening of Lamtoro leaf extract was also conducted using the HPLC instrument to determine the presence of quercetin, a secondary metabolite belonging to the flavonoid group. From the analysis results depicted in Figure 1, it is evident that Lamtoro leaf extract positively contains quercetin, as indicated by the peak appearing at the same retention time as the quercetin standard. Peak from the quercetin standard solution appears at 2.582 minutes. Meanwhile, the Lamtoro leaf extract sample produces a similar peak at 2.554 minutes, indicating the presence of quercetin compounds in the Lamtoro leaf extract. Research conducted by Negi et al. [18] stated that quercetin has proven antifeedant activity against Spodoptera Litura larvae, showing potential as a botanical insecticide.

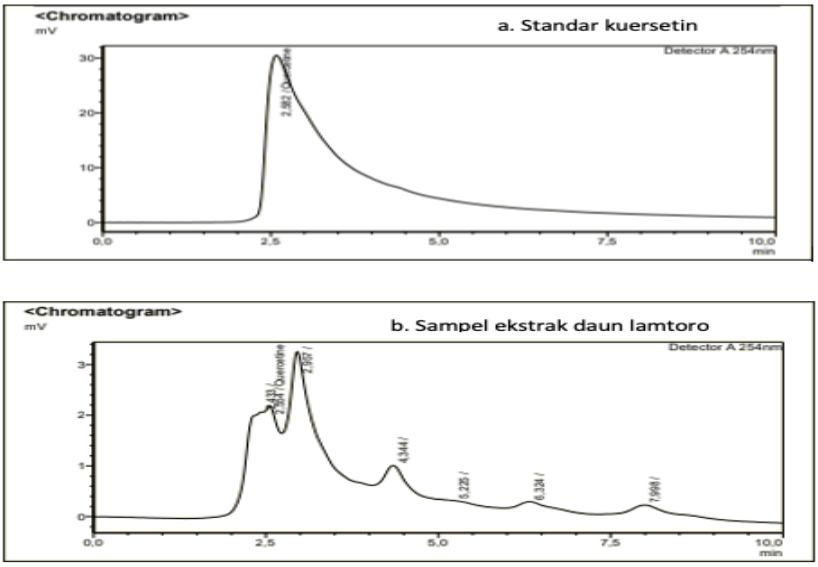


Fig. 1: HPLC screening results for quercetin compounds in lamtoro leaf extract

➢ *Screening Results with GC-MS/MS*

Ethanol extract of Lamtoro leaves was also screened using GC-MS/MS to identify volatile secondary metabolites such as fatty acids, terpenoids, alcohols, and their derivatives. The analysis results for the Lamtoro leaf extract dissolved in acetone present a set of spectra shown in Figure 2. The emerging spectra are then compared for similarities with active compounds stored in the NIST 17.1 library.

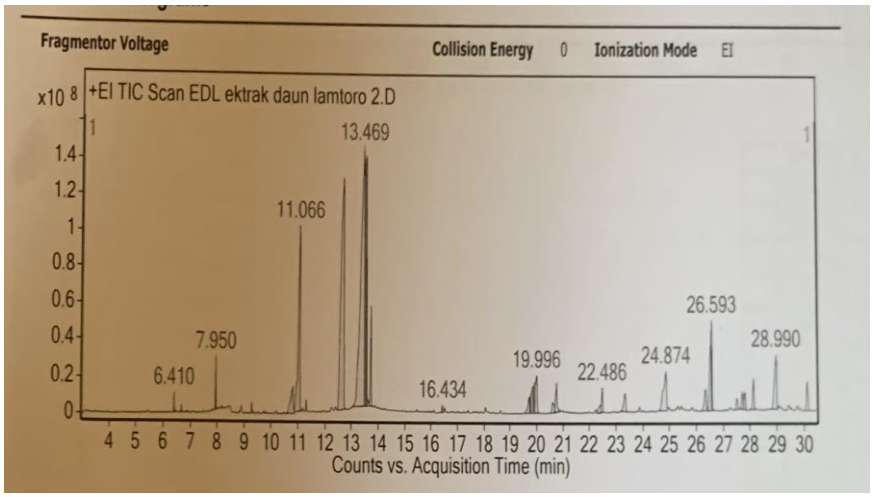


Fig. 2: Spectra of lamtoro leaf extract in acetone as a result of GC-MS/MS analysis.

➢ *Flavonoid Compound Levels in Lamtoro Leaf Extract*

Based on the results of phytochemical screening using the phytochemical tube method, it is known that lamtoro leaf extract tested positive for flavonoids. Flavonoid compounds are known for their abilities as antibacterial agents [19], antioxidants, anti-radicals, and anti-inflammatory agents [20]. They function by disrupting the body's defense system, lipid oxidation, and protein oxidation [21]. According to Robinson, flavonoid compounds have the ability to regulate growth, photosynthesis, as well as exhibit antimicrobial and antivirus properties [22]. Flavonoid compounds consist of two aromatic rings, and this system can exhibit strong absorption bands in the UV-Vis wavelength range, with quercetin used as the standard [20]. To determine the flavonoid content in the sample, testing was conducted

using a UV-Vis spectrophotometer with the colorimetric method, which involves adding AlCl3 to the sample to produce a stable yellow color due to the reaction between flavonoids and AlCl3 [17]. From the analysis results, it is revealed that lamtoro leaf extract contains 67.0498 mg Quercetin Equivalent/Gram of Lamtoro Leaf Extract, equivalent to 6.7%.

B. Encapsulation of Lamtoro Leaf Using Chitosan and Sodium Tripolyphosphate Crosslink Agent

The microcapsules produced in this study underwent characterization, including analysis of encapsulation efficiency, particle size, yield, surface morphology, and functional groups to assess the success of the encapsulation process that has been conducted.

➢ *Encapsulation Efficiency*

Encapsulation efficiency is a measure indicating the ability of a matrix to encapsulate an active ingredient. This value is the result of comparing the active ingredient that is encapsulated in the matrix with the total amount of active ingredients added to the system.

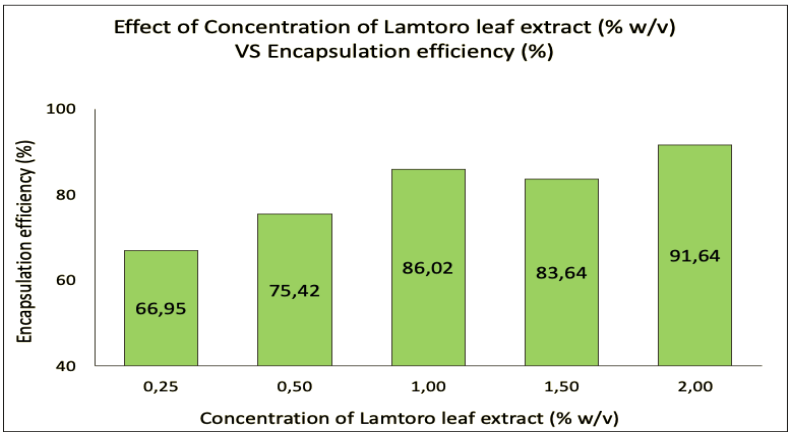


Fig. 3: Effect of Concentration of Lamtoro Leaf Extract (%b/v) on Encapsulation Efficiency (%)

In Figure 3., it can be observed that the increase in the concentration of lamtoro leaf extract is directly proportional to the increase in encapsulation efficiency of the microcapsules. When lamtoro leaf extract is added at a concentration of 0.25% to the system, the resulting encapsulation efficiency is only 66.95%. As the concentration of the extract in the system increases, the encapsulation efficiency values also increase, reaching a maximum value of 91.64% when 2% of lamtoro leaf extract is added to the system. This result is consistent with a study by Prasetyaningrum et al. [23], where an increase in the concentration of lemongrass in the solution led to higher encapsulation efficiency ranging from 74% to 83%.

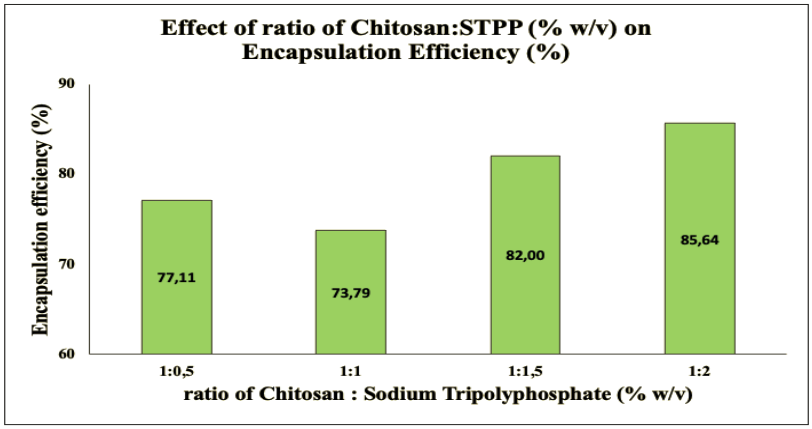


Fig. 4: Effect of ratio of Chitosan: NaTPP (% w/v) on Encapsulation Efficiency

The comparison of chitosan to NaTPP (% w/v) in the system has a direct impact on encapsulation efficiency, as shown in Figure 4. Increasing the crosslink agent (NaTPP) concentration relative to chitosan tends to enhance encapsulation efficiency. The highest encapsulation efficiency, 85.64%, is achieved at a chitosan:NaTPP ratio of 1:2. This is attributed to the increased ionized negative phosphate groups with higher NaTPP concentrations, leading to more crosslinking interactions with positively charged amino groups from chitosan. The formation of chitosan:NaTPP complexes enhances the adsorption capacity of the active ingredient.

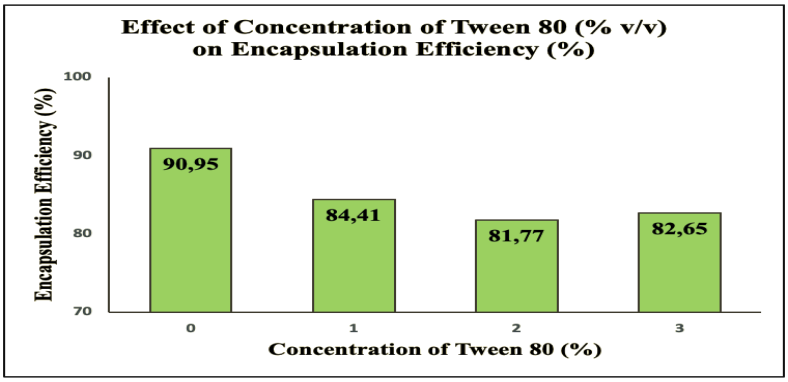


Fig. 5: Effect of Concentration of Tween 80 (% v/v) on Encapsulation Efficiency (%)

Furthermore, the study also examined the influence of adding Tween 80 on encapsulation efficiency. In this research, the highest encapsulation efficiency, reaching 90.95%, was achieved when the system did not receive additional Tween 80 as shown in fig. 5. This condition arises due to the non-solubility of flavonoids in water, hindering the easy diffusion of the active ingredient into the acetic acid solution during the encapsulation process. With the addition of 1-3% Tween 80 to the system, the solubility of the active ingredient in the system increases, resulting in more active ingredients not being encapsulated due to diffusion into the solution.

➢ *Microcapsule Yield from Encapsulation*

Yield is the ratio of the weight of microcapsules obtained after the drying process to the total weight of all microcapsule-forming materials, including lamtoro extract, chitosan, and crosslink agent, calculated based on dry weight.

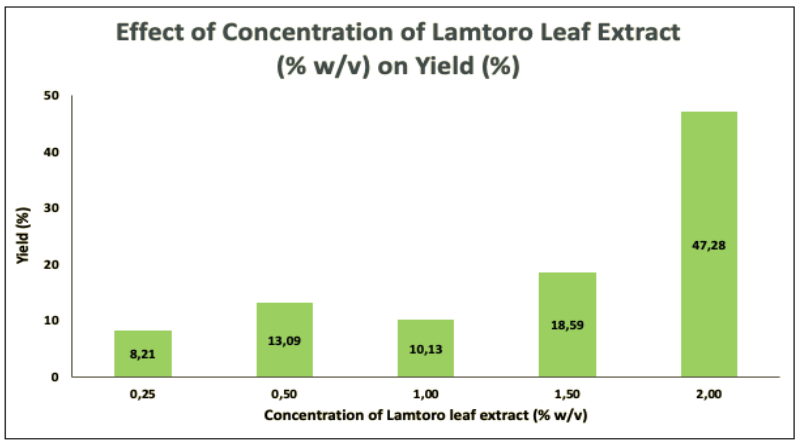


Fig. 6: Effect of Concentration of Lamtoro Leaf Extract (% w/v) on Microcapsule Yield (%)

From the research results, it is evident that the greater the amount of lamtoro leaf extract in the system, the higher the yield of microcapsules obtained as shown in figure 6. The highest percentage yield, 47.28%, was obtained at a concentration of 2% extract in the system. This implies that the more lamtoro leaf extract present in the system, the more extract is encapsulated, resulting in a higher yield of microcapsules.

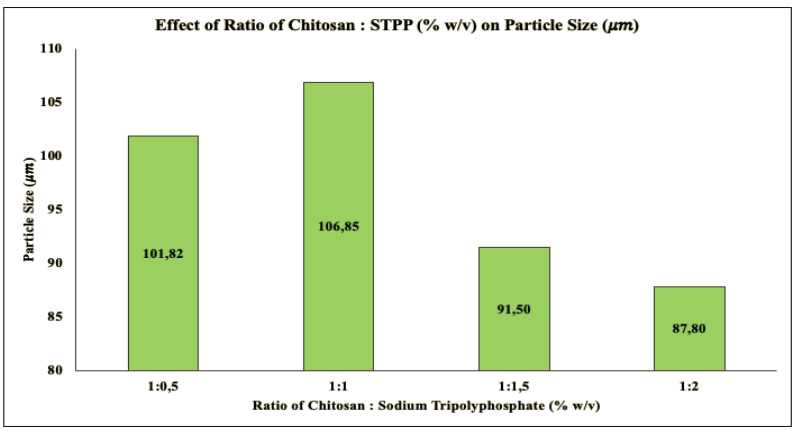


Fig. 7: Effect of ratio of Chitosan : STPP (% w/v) on Microcapsule Yield (%)

Figure 7 conducted the influence of the ratio of coating materials to crosslink agent on the microcapsule yield is that the more crosslink agent in the system, the higher the yield of microcapsules obtained. This is because with an increase in the number of phosphate ions in the solution, crosslinking with chitosan increases. This condition affects the increased absorption of the active ingredient, resulting in a higher yield of microcapsules.

Meanwhile, as shown in figure 8, the addition of 3% Tween 80 to the system resulted in the highest microcapsule yield, reaching 83.97%. The addition of Tween 80 to the solution can aid in the emulsification process of bioactive compounds present in lamtoro leaf extract, improving the encapsulation process and increasing the yield of microcapsules.

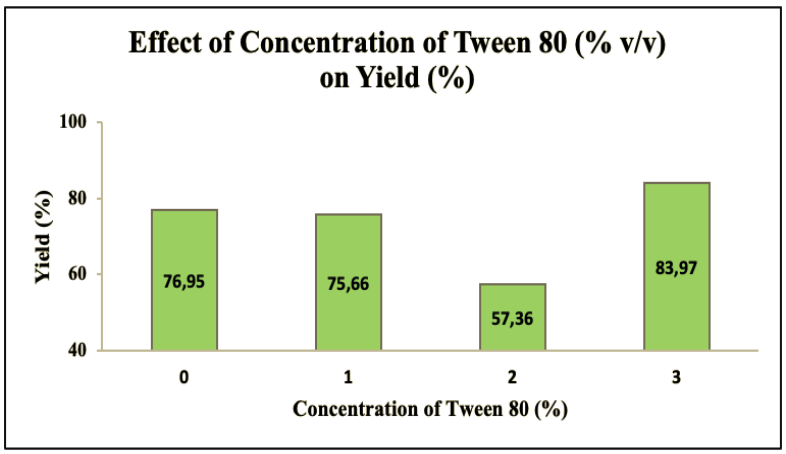


Fig. 8: Effect of Concentration of Tween 80 (%) on Microcapsule Yield (%)

➢ *Particle Size Distribution*

The size of the particles produced is a crucial parameter as smaller particles are expected to be more effective in the active ingredient delivery system. In this study, the encapsulation of lamtoro leaf extract using chitosan through the ionic gelation method resulted in micro-sized particles ranging from 27.6 µm to 106.8 µm as shown in figure 9. The increase in particle size from nano to micro after the addition of lamtoro leaf extract aligns with the findings of Keawchaoon and Yoksan [11], which involved encapsulating carvacrol. The trend of increasing particle size is attributed to the growing amount of active ingredients in the system, causing an increase in the number of encapsulated active ingredients and enlarging the diameter of microcapsule particles.

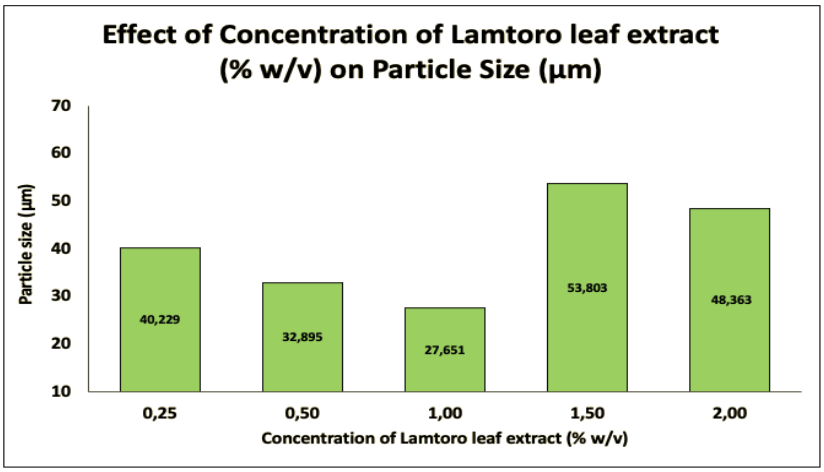


Fig. 9: Effect of Concetration of Lamtoro Leaf Extract (%w/v) on particle size (µm)

Regarding the variable of lamtoro leaf extract concentration, it appears that the extract concentration in the system does not significantly affect the particle size. At a concentration of 0.25%, the particle size is 40 µm, and it gradually decreases at extract concentrations of 0.5% and 1%, measuring 32.9 µm and 27.6 µm, respectively. However, when the lamtoro leaf extract concentration is increased to 1.5%, the particle size dramatically increases to 53.8 µm, and then the average particle size slightly decreases at an extract concentration of 2%, measuring 48.4 µm. The results of particle size distribution are similar to a study by Mondéjar-López et al [6], where at a 1:0.75 ratio, the average particle size is the largest, but the particle size decreases at a chitosan:garlic extract ratio of 1:1. The increase in particle size with the addition of extract to the system is due to the growing amount of encapsulated extract in chitosan, leading to an increase in particle size. In this study, the smallest particle size is achieved when 1% extract is added to the system, measuring 27.6 µm.

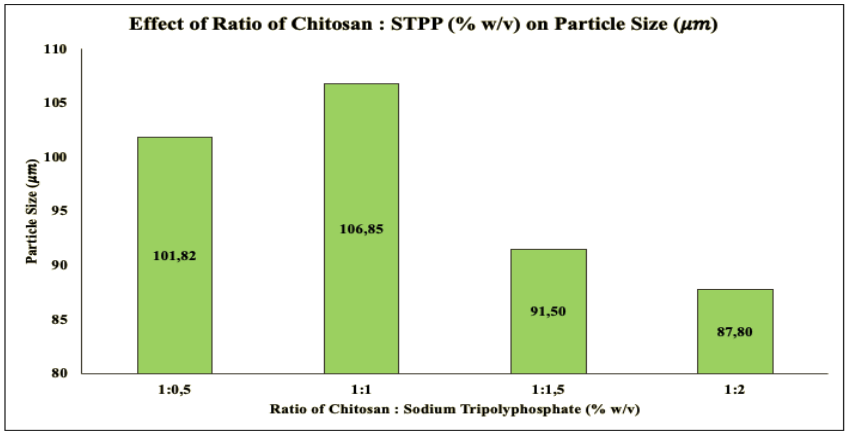


Fig. 10 Effect of ratio of Chitosan : STPP (% w/v) on Particle Size (μm)

Meanwhile, in the variation of the chitosan:Sodium Tripolyphosphate concentration ratio, particle size distribution ranging from 87.8 μm to 106.8 μm is observed, as shown in Figure 10. An excess of chitosan in the system will increase the particle size because the dispersed polymer chain folding structure will become larger and more abundant.

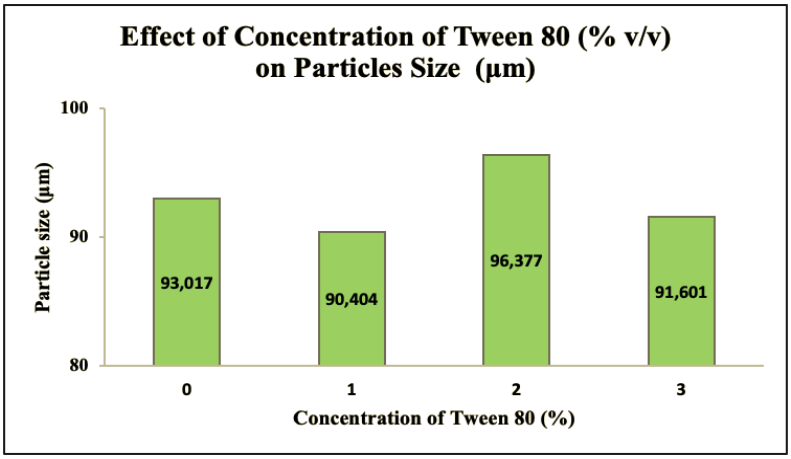


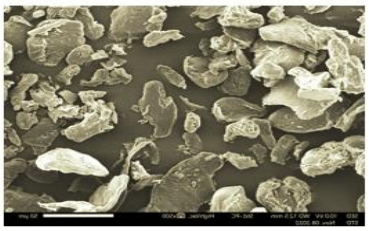
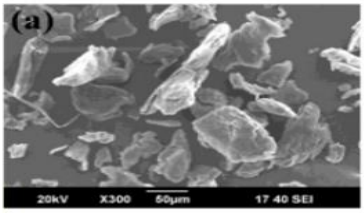
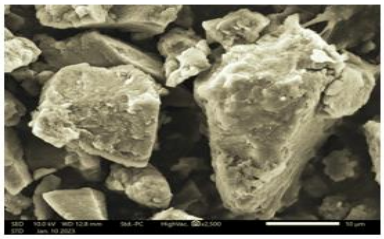
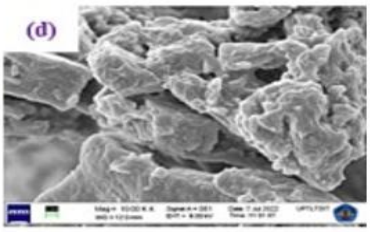
Fig. 11 Effect of Tween 80 Concentration (%) on Particle Size (μm)

The addition of Tween 80 in the microencapsulation process aims to reduce particle aggregation, with the expectation of producing smaller particle sizes. The initial experiment without Tween 80 resulted in particles with a size of 93 μm. Subsequent additions of 1%, 2%, and 3% Tween 80 were made. The addition of 1% Tween 80 successfully reduced the particle size to 90.4 μm, indicating that Tween 80 can decrease surface tension, increase surface area, and prevent aggregation among the formed particles. Meanwhile, with the addition of 2% Tween 80, the particle size slightly increased to 96.4 μm, and at a concentration of 3% Tween 80, the particle size decreased to 91 μm. The differences in particle size among the various variations are not significantly notable, with a relatively narrow range between 90.4 μm and 96 μm. The smallest particle size in this study was achieved with the addition of 1% Tween 80 as shown in figure 11.

➢ *Scanning Electron Microscope*

Microencapsulation of lamtoro leaf extract using chitosan and the crosslink agent sodium tripolyphosphate was carried out using the ionic gelation method, at room temperature with a stirring speed of 500 rpm. The surface morphology of the microcapsules needs to be analyzed to determine the morphological differences between pure chitosan and chitosan after encapsulation of lamtoro leaf extract. Table 2 represent SEM image of pure chitosan with 500 times magnificient. Pure chitosan relatively irregular and smooth, this result similar with the study by Kim et al [24]. Surface morphology of chitosan loaded lamtoro leaf extract is rougher and harder. This chitosan rough surface indicate that the lamtoro extract has been entrapped in the matrix [23]. The structure of microcapsule relatively irregular maybe because of agglomeration of the particles, with some sperichal structure . This result similar with the microcapsule of andaliman in chitosan-gelatin by Gea et al [25].

Table 2: SEM image of chitosan and chitosan-lamtoro leaf microcapsules

Our research result	Reference
 Chitosan (500 x)	 Kim et al
 Chitosan-lamtoro leaf extract microcapsules (2500 x)	 Gea et al

➢ *Fourier Transform Infrared Spectroscopy*

The Fourier Transform Infrared Spectroscopy (FTIR) analysis serves as a method to identify functional groups in a compound, allowing insight into the composition of pure chitosan, lamtoro leaf extract, and chitosan-lamtoro leaf extract microcapsules. This analysis also aids in confirming the success of the encapsulation process by examining the functional group compatibility between the microcapsules and their constituents. According to figure 12, the FTIR analysis of pure chitosan powder reveals a broad spectrum between the wavelength range of 3700-2800 cm^{-1}, indicating O-H stretching vibration from carbohydrates overlapping with N-H stretching [16]. Peaks at wavelengths such as 1650.59 cm^{-1} (C=O stretching of amide I, indicating N-acetylglucosamine structure), 1557.79 cm^{-1} (N-H bending indicating N-acetylation residue), 1394.67 cm^{-1} (N-H stretching, amide III), and 1016.32 cm^{-1} (C-O stretching) are observed [16].

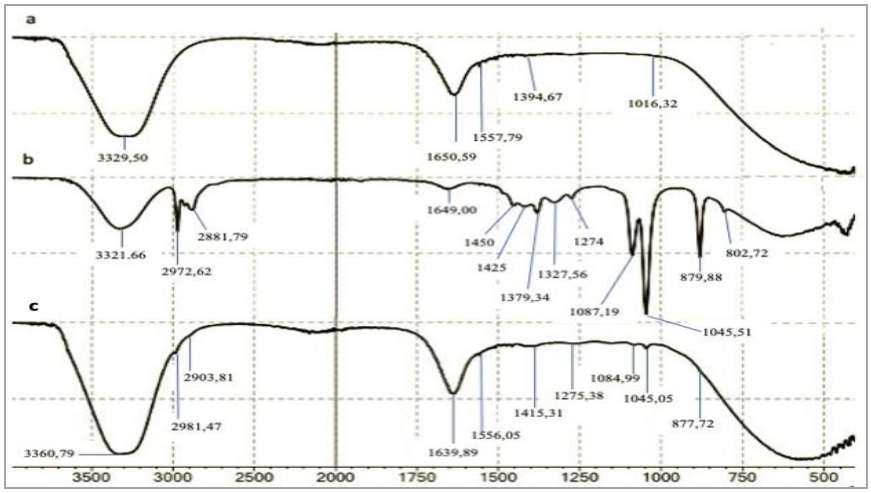


Fig. 12: Comparison of FTIR spectra for pure chitosan (a), lamtoro leaf extract (b) and chitosan-lamtoro leaf extract (c)

The FTIR spectrum of lamtoro leaf extract exhibits a broad spectrum in the wavelength range of 3600-3000 cm^{-1} due to stretching vibrations of -OH groups, 2972.62 cm^{-1} and 2881.79 cm^{-1} indicate stretching of asymmetric and symmetric CH2 groups found in tannin compounds. Peaks at 1649.00 $cm^{-1}$and 1450 cm^{-1} represent C=O stretching, while the peak at 1379.34 cm^{-1} and 1327.56 cm^{-1} is attributed to -OH bending vibrations. The presence of quercetin-derived O-H groups is evident at 1274 cm^{-1}. Peaks at 1087.19 cm^{-1} denote C-O stretching, 1045.51 cm^{-1} indicates C-N stretching, 879.88 cm^{-1} and 802.72 cm^{-1} indicate Ar-H group substitution.

In the FTIR spectrum of chitosan-lamtoro leaf extract microcapsules, peaks identical to chitosan are observed at 3360.79 cm^{-1}. Meanwhile, several peaks identical to lamtoro leaf extract are also present at 2981.47 cm^{-1}, 2903.81 cm^{-1}, 1415.31 cm^{-1}. Peak at 1275.38 cm^{-1} shows the P-O group from sodium tripolyphosphate, indirectly

indicating successful crosslinking between chitosan and TPP [16]. Peaks at 1084.99 cm^{-1} (C-O stretching), 1045.05 cm^{-1} (C-N stretching), and 877.72 cm^{-1} (Ar-H substitution) suggest the presence of tannin, flavonoid, and phenolic compounds from lamtoro leaf extract. These peaks collectively indicate successful interaction between chitosan and lamtoro leaf extract, confirming the effectiveness of the encapsulation process.

➢ *Release Study of Active Ingredient*

This active ingredient release study is conducted to assess the ability of chitosan crosslinked with sodium tripolyphosphate to protect the encapsulated active ingredient. The release mechanism of the active ingredient is influenced by factors such as desorption on the encapsulant surface, diffusion, and erosion through the matrix pores or polymer wall [26]. The microcapsules used for the active ingredient release study are derived from the formula with the most optimal results, namely 2% lamtoro leaf extract, chitosan:NaTPP ratio (1:2), without Tween 80. In this research, the release time is observed for 24 hours in a buffer solution with pH 1.3 (0.1 N HCl), pH 4.3, and pH 7.2 (Phosphate Buffer). The percentage of active ingredient released at specific time intervals can be seen in the graph below.

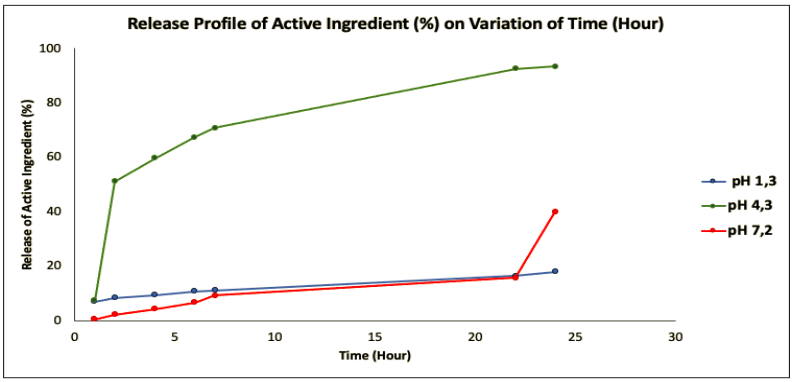


Fig. 13: Active Ingredient Release Graph Against Time

At pH 7.2 and 1.3, the released active ingredient over 24 hours did not reach 50%, only amounting to 39.96% and 17.90%, respectively. These results align with the study conducted by Soltanzadeh et al. [27] on the release of encapsulated lemongrass oil by chitosan nanoparticles. In that research, only 32% of lemongrass oil was successfully released in a pH 7.4 medium over 1440 minutes or 24 hours. Meanwhile, at pH 3, a total of 48% of the active ingredient successfully released into the medium.

In Figure 13, it can be observed that in the first hour, the release of the active ingredient at pH 1.3 and 4.3 (acidic conditions) reached 7%, much higher than the release at pH 7.4, which was only 0.45%. This is likely due to instantaneous diffusion of the active ingredient attached to the chitosan surface. The release of the active ingredient at pH 4.3 experienced a significant spike between 1-2 hours and continued to increase, reaching 93.45% at 24 hours. The burst release in the first 2 hours occurred because of instant diffusion of weakly bound active ingredients on the chitosan surface. The highest release of the active ingredient at pH 4.3 is due to the acidic environment causing chitosan to undergo amino group protonation, resulting in swelling and a change in its structure to a gel, facilitating the easy release of the trapped active ingredient into the medium [27]. Meanwhile, at pH 7.4, the release of the active ingredient occurred slowly and only experienced a spike after 24 hours. This condition is because of the strong complex formation between chitosan and sodium tripolyphosphate, making it challenging for the buffer solution to penetrate the chitosan matrix [28]. After 24 hours, the buffer solution likely succeeded in breaking the complex bonds, leading to a faster release of the active ingredient.

Based on the above active ingredient release profile, it is evident that chitosan has the capability to effectively protect the active compounds present in lamtoro leaf extract over an extended period. This protection results in a more controlled release of lamtoro leaf extract, which is highly beneficial for its further applications with controlled, prolonged release.

C. Results of Rice Seed Germination Test

The microcapsules produced from the most optimal formulation were then applied as a seed soaking agent for rice. The purpose of seed soaking before planting is to enhance seed germination and prevent fungal growth during seedbed preparation. Seed germination is the ability of a seed to grow normally in a suitable environment. The results of germination between the control, where seeds were directly sown without soaking, and seeds soaked in various microcapsule variations showed no significant differences. Chitosan-lamtoro leaf extract microcapsules have very low solubility in water, so the diffused active ingredient in the medium is absorbed by the seeds at concentrations that are still too low to significantly impact seed germination. However, seeds soaked in microcapsules dispersed in pH 4.3 buffer exhibited the highest germination rate, reaching 68%. This is because chitosan microcapsules easily dissolve in acidic pH, allowing the active ingredients to readily diffuse into the medium and be absorbed by the seeds. This indicates that chitosan and lamtoro leaf extract can indeed enhance the germination of rice seeds. Seeds soaked in chitosan nanoparticles also exhibited a higher germination rate than the control, reaching 65%. This is attributed to the small particle size, enabling chitosan nanoparticles to enter the seed pores and enhance seed germination.

Table 3: Germination capacity of rice seeds with various types of soaking materials

Material type	Concentration	Solvents	Germination Strength (%)
Control	-	-	63 %
K-EDL Microcapsules	1 mg/ml	Ultrapure Water	63 %
K-EDL Microcapsules	5 mg/ml	Ultrapure Water	63 %
K-EDL Microcapsules	10 mg/ml	Ultrapure Water	62 %
K-EDL Microcapsules	1 mg/ml	Buffer pH 4,3	68 %
Chitosan nanoparticles	1 mg/ml	Ultrapure Water	65 %

IV. CONCLUSIONS

Based on the results of the conducted research, it can be concluded that chitosan-lamtoro leaf extract microcapsules were successfully produced using the ionic gelation method. In the fabrication of chitosan-lamtoro leaf extract microcapsules, the concentration of Lamtoro leaf extract is directly proportional to the encapsulation efficiency and microcapsule yield but does not affect the particle size. The optimum concentration of Lamtoro leaf extract is 2%, yielding an encapsulation efficiency of 91.64%, a yield of 47.28%, and a microcapsule particle size of 48.363 µm. The concentration ratio of chitosan: NaTPP is directly proportional to the encapsulation efficiency and microcapsule yield but inversely proportional to the microcapsule particle size. The optimal formula, which uses a chitosan: NaTPP ratio of 1:2, results in an encapsulation efficiency of 85.04%, a yield of 81.09%, and a particle size of 87.804 µm. The concentration of Tween 80 is inversely proportional to the encapsulation efficiency, has no effect on the yield, and is not related to the particle size. The optimum formula in the fabrication of chitosan-lamtoro leaf extract microcapsules is without the addition of Tween 80, resulting in an encapsulation efficiency of 90.95%, a yield of 76.95%, and a particle size of 93.017 µm. The application of microcapsules as a seed soaking agent had little effect in seed germination. Further investigation is needed to explore the impact of variations in the crosslinking agent and higher extract concentrations on the characteristics of microcapsules and the active ingredient release profile.

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