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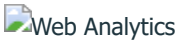

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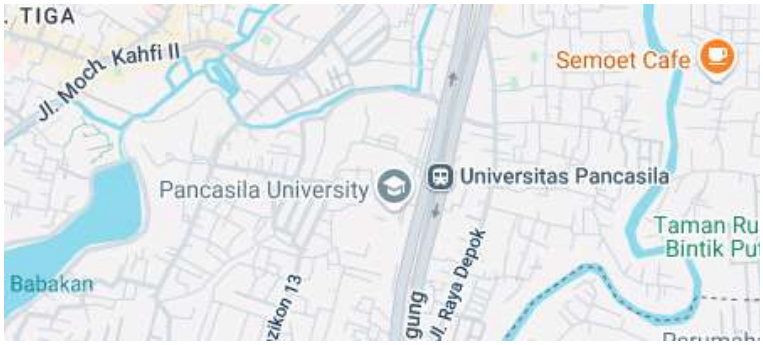

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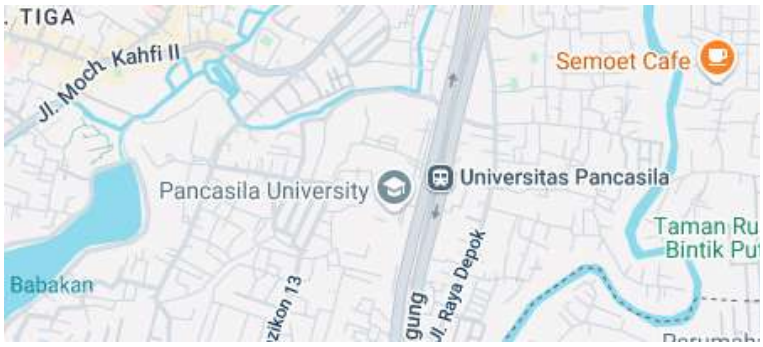

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Characteristic and therapeutic effect of lemongrass leaf encapsulation with a different ratio of chitosan and NaTPP

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ABSTRACT: The lemongrass plant (*Cymbopogon citratus*) in Indonesia has great potential as a natural ingredient for medicines because it contains various compounds such as neral, citral, geranial acetate, flavonoids, and tannins. Previous research has demonstrated various pharmacological activities of kitchen lemongrass leaves, including antibacterial, antifungal, and anti-inflammatory properties. Encapsulation technology in the form of chitosan-NaTPP nanoparticles is used to maintain the activity of kitchen lemongrass leaf compounds. This research aims to determine the physical characteristics of the encapsulation of kitchen lemongrass leaf extract with several comparisons of chitosan and NaTPP by testing PSA (particle size analysis test), FTIR (functional group analysis test), viscosity, and stability, including pH and turbidity tests. Encapsulation with a 1:1 ratio of chitosan and NaTPP has a particle size of 524 nm and a PdI value of 0.481, classified as monodisperse (PdI <0.7). The pH value ranges from 6.46 to 7.30, and the viscosity value was 2.134–2.169 cP, which is still within mouthwash standards. The turbidity test showed stable encapsulation results. Therefore, encapsulation with a 1:1 ratio of chitosan and NaTPP is the most optimal choice and has potential for developing the therapeutic effect of mouthwash.

KEYWORDS: Chitosan; encapsulation; lemongrass leaves; mouthwash; NaTPP.

INTRODUCTION

Indonesia is rich in natural plant sources as raw materials for medicines. One plant that is widely used is the kitchen lemongrass leaves. Many researchers are taking advantage of technological advances and making various efforts to improve the quality and trust in the benefits of natural sources, one of which is kitchen lemongrass leaves [1]. *Cymbopogon citratus*, commonly called kitchen lemongrass, has a larger lower stem, is white, and does not smell too sharp. The part that is often used as a kitchen spice is the young inner part. Lemongrass plays a role as an aromatic spice in cooking and is also used as a complement to chili sauce. These plants can both produce essential oils, but in Indonesia, only citronella is widely processed as an essential oil producer [2]. The results of various studies show that citronella leaves are rich in essential oils and contain neral (31.55%), citral (26.1%), and geranial acetate (2.27%). This ingredient is often used as an aromatherapy oil [3]. This plant also contains active compounds, namely flavonoids, saponins, tannins, phenols, aldehydes, phenolic acids, and terpenoids. Studies also show that lemongrass leaves have various pharmacological properties, such as antibacterial, antifungal, and anti-inflammatory, especially when used as a mouthwash [4].

One way that can be used to protect secondary metabolic activity in lemongrass leaf extract is through encapsulation technology, which is a technique for covering secondary metabolism to protect it from external influences. Encapsulation is divided into micro and nanoparticle forms. Applications of food nanotechnology show an increasing trend. This technology offers advantages in increasing secondary metabolic bioavailability, controlling the release of active compounds, and improving sensory properties [5]. Nanoparticles are solid colloidal particles with a diameter of 1-1000 nm and are materials that can be used as encapsulated active compounds [6]. The active compound of lemongrass leaves has poor stability, so it is encapsulated with horn beetle chitosan to increase its stability, such as antioxidant, anti-cancer, anti-inflammatory, anti-fungal, and antibacterial [7].

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Chitosan is a natural polysaccharide that is non-toxic and easily biodegradable in delivery systems because it has properties as a matrix. Chitosan also forms gels in acidic conditions and has a structure similar to chitin [8]. Chitosan is a compound of poly (N-amino-2-deoxy-β-D-glucopyranose) or glucosamine resulting from the deacetylation of chitin/poly (N-acetyl2-amino-2-deoxy-βDglucopyranose) [9].

Previous studies by Purwandari showed that moringa leaf extract was obtained by repeated ultrasonication of 1x15, 3x15, and 5x15 minutes, frequency 24 kHz, and using 96% alcohol solvent. The encapsulation process was carried out with three variable ratios of the amount of chitosan, Na-TPP added to 10 mL of moringa leaf extract solution, namely 5:1, 2:1 and 1:1. The results of the flavonoid test as quercetin using a UV-Vis spectrophotometer showed that the quercetin content in moringa leaf extract resulting from 5x15 minute ultrasonication was 12.146%, Particle Size Analyzer (PSA) characterization showed encapsulated particle sizes of 170.3 and 209.7 nm with a polydispersion index <0.5, nanoencapsulation of moringa leaf extract has been produced. There has been an increase in the antioxidant activity of moringa leaf extract after encapsulation using chitosan-NaTPP 2:1, namely from 61.4% to 81.2% [9].

Based on the background above, this research was conducted to determine the physical characteristics of chitosan encapsulation of kitchen lemongrass leaf extract with several comparisons of chitosan and NaTPP, which will determine the activity of the encapsulated particles as antibacterial, anticancer, and antioxidant.

▪ MATERIALS AND METHODS

Materials

This study was conducted with lemongrass leaf extract (Bogor, Indonesia), 70% ethanol (Merck, Germany), 1% acetic acid (Merck, Germany), distilled water (Merck, Germany), chitosan *Xylotrupes gideon* (Bogor, Indonesia), NaTPP (Merck, Germany), and Tween 80 (Merck, Germany).

Kitchen lemongrass leaf extraction

Lemongrass leaf simplicia was extracted using the maceration method by soaking samples of kitchen lemongrass leaves in 70% ethanol with a ratio of 1:10 for 3 × 24 hours at a temperature of 27 ^{o}C. The solution will be filtered using Whatman filter paper and spun using an evaporator machine at 50-60 ^{o}C.

Encapsulation of lemongrass leaf extract

The nano chitosan encapsulation stage was achieved by dissolving 40 mL of chitosan in 1% acetic acid and adding 100 mL of distilled water. Lemongrass leaf extract is dissolved using distilled water. Then, the extract solution was mixed into the chitosan solution. Stirring was carried out at a speed of 2500 rpm for 2 hours. Next, tripolyphosphate was given drop by drop. Finally, 0.1 mL of Tween 80 was added to the solution. In making the encapsulation, a comparison was made between the extract and chitosan, presented in Table 1.

Table 1. Encapsulation comparison.

Group (ratio chitosan : NaTPP)	Chitosan (mg/mL)	NaTPP (mL)	Lemongrass (mL)	Tween 80 (mL)
Group 1 (1 : 1.0)	0.57	0.57	2	0.1
Group 2 (1 : 0.9)	0.57	0.514	2	0.1
Group 3 (1 : 0.8)	0.57	0.468	2	0.1
Group 4 (1 : 0.7)	0.57	0.429	2	0.1

Particle size analysis test

An encapsulated sample of lemongrass leaf extract in liquid form was prepared and dropped into a cuvette to which sufficient distilled water was added as a solvent. Then, it was put into the PSA apparatus, and the temperature was set to 25 °C. The results of the sample particle size distribution will appear on the monitor.

FTIR test

The FTIR (Fourier Transform Infrared Spectroscopy) test was used to analyze the functional groups contained in the sample. The chemical interactions between NaTPP, chitosan, and kitchen lemongrass leaf extract were analyzed using an FTIR spectrophotometer at wave numbers 4000 – 400 cm^{-1}. Analysis was carried out using the Shimadzu Prestige-21 FTIR tool. A total of 1 mL of encapsulated sample for each formulation was frozen using a freeze dryer until it reached a temperature of -80 °C. Then, the sample was put into the tool and analyzed. Next, the results are displayed on the monitor.

Stability test

Turbidity test

Turbidity test by visible observation. This observation aims to see the occurrence of material aggregation within 2 weeks. The measurement results were color and turbidity.

pH determination test

Acid measurements were carried out using a Mettler Toledo pH meter. First, prepare the sample whose acid level you want to measure (place it in a container). Next, the electrode from the pH meter was inserted into a container containing the solution to be tested. When immersed in the solution, the number scale will move randomly. Wait until the number stops and does not change. The results will be visible on the monitor.

Viscosity test

The viscosity test was carried out through measurements using the ViscoQC 100 tool Anton Paar (rotational viscometer). The time is set to 1 minute, the torque is 56%, and the speed is 200 rpm. The results displayed on the monitor screen are recorded. Viscosity measurements were carried out on days 1, 7, and 14.

▪ RESULTS

Particle size analysis test (PSA)

The PSA measurements showed that the encapsulation group with a 1:1 ratio of chitosan and NaTPP had the smallest size of 524.0 nm compared to the other comparison groups. The comparison group for chitosan and NaTPP encapsulation, which has the largest to smallest size, was the ratio of chitosan and NaTPP encapsulation 1:0.7, 1:0.8, 1:0.9, and 1:1, with particle sizes of 1145 nm, 934.2 nm, 614.5 nm, and 524 nm, respectively, as shown in Table 2. Particle measurement results, the ratio of chitosan and NaTPP encapsulation was 1:0.8, 1:0.9, and 1:1, which fall into nano-sized particles, while the encapsulation ratio of chitosan and NaTPP 1:0.7 with a size of more than 1000 nm does not meet the requirements for particle preparation.

Polydispersity Index (PdI) is an index of the heterogeneity of a collection of particles. The average PdI value of the encapsulation ratio of chitosan and NaTPP was 1:1, 1:0.9, and 1:0.8, with PdI values of 0.481, 0.695, and 0.687, respectively. PdI values from a 1:1 chitosan encapsulation ratio of 1:0.9 and 1:0.8 have a PdI value of <0.7. According to Chasana et al., particles can be classified into monodisperse groups (PdI value <0.7) and polydisperse large size distribution (PdI value>0.7) [10]. A PdI value <0.7 indicates good particles because it is in the middle range of the polydispersity index. For encapsulation, the ratio of chitosan and NaTPP 1:0.7 has a value of 0.747, which indicates that the sample has a part with a large particle size and is not homogeneous.

Table 2. Particle size and polydispersity index.

Group (ratio chitosan : NaTPP)	Particle size (nm)	PdI
Group 1 (1 : 1.0)	524.0	0.481
Group 2 (1 : 0.9)	614.5	0.695
Group 3 (1 : 0.8)	934.2	0.687
Group 4 (1 : 0.7)	1145	0.747

FTIR test

The graphical image of the analysis of the FTIR test results showed that several functional groups were identified in the four samples (Figure 1). In the chitosan extract sample, there were absorption peaks in the wave number area 3425 cm^{-1} (O-H stretching vibrations overlap with N-H), 1627.99 cm^{-1} (C=O Amide I stretching vibrations), 1579.77 cm^{-1} (C=O Amide I stretching vibrations). NH_2 bending in Amide II). The four chitosan encapsulation preparations experienced a slight shift in the absorption peak at the amide I carbonyl group, namely around the number area 1642.46 cm^{-1} for the 1:1 formulation, 1640.53 cm^{-1} for the 1:0.9 formulation, 1638.60 cm^{-1} for the 1:0.8 formulation, and 1645.35 cm^{-1} for the 1:0.7 formulation. Furthermore, there is also a shift in N-H bending vibrations in the area of 1566.27 cm^{-1} for the 1:1 formulation, 1564.34 cm^{-1} for the 1:0.9 formulation, 1550.83 cm^{-1} for the 1:0 formulation 8, and 1563.37 cm^{-1} for the 1:0.7 formulation. Besides that, there was an absorption peak of the P-O group in the number area 917.19 cm^{-1} for the 1:1 formulation, 919.12 cm^{-1} for the 1:0.9 formulation, and 921.05 cm^{-1} for the 1:0.8 formulation. Next, the formulation 1:0.7 was located in the number area 922.98 cm^{-1}.

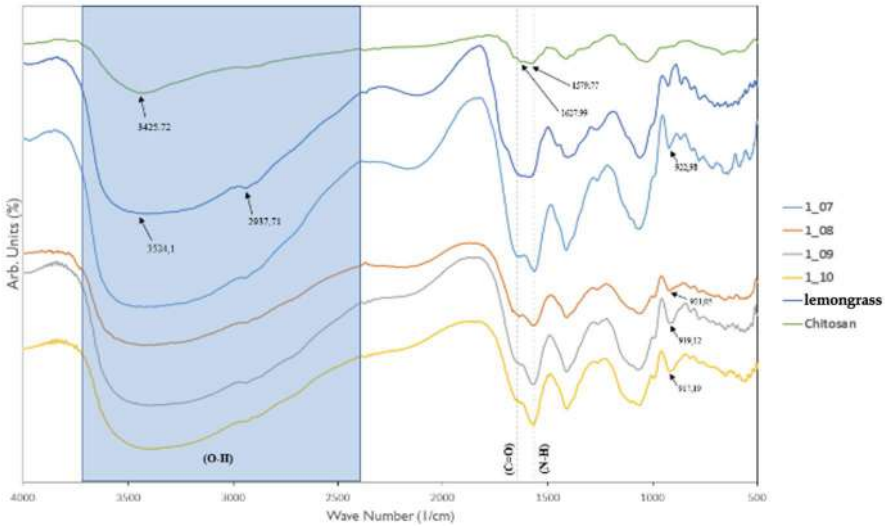

Figure 1. FTIR analysis results.

pH determination

The results of the pH normality test of encapsulated chitosan and NaTPP in trapping lemongrass leaf extract in a ratio of 1:1, 1:0.9, 1:0.8, and 1:0.7, with p value>0.05. The normality test results were followed by a repeated ANOVA test to see whether there was a difference in pH values on the first day and the seventh day. The parametric test results showed no significant difference in pH values on the first and seventh days (p>0.05). The pH test results can be seen in Table 3.

Encapsulation of chitosan and NaTPP in entrapping lemongrass leaf extract in a ratio of 1:1; 1:0.9; 1:0.8; and 1:0.7 on the first day of pH observation showed that the encapsulation group with a ratio of 1:0.8 was significantly different (p<0.05) from the other encapsulation groups, which were shown by the lowest pH value, namely 5.54 ±0.17. On the seventh day, the pH of the 1:1 ratio encapsulation group was not significantly different from the 1:0.7 ratio with a p value of 0.06, but was significantly different from the 1:0.8 and 1:0.9 ratio encapsulation groups (p<0.05). Each formulation has an increase and a decrease in the pH value. Encapsulation 1:1, 1:0.8, and 1:0.7 experienced an increase in pH on the seventh day. Meanwhile, encapsulation 1:0.9 saw a decrease in pH on the seventh day. Changes in pH values can be observed in Table 3.

Table 3. pH determination test.

Group (ratio chitosan : NaTPP)	pH level		pH of commercial mouthwash [11]	Description
	Day 1	Day 7		
Group 1 (1 : 1.0)	6.46±0.14	7.30±0.14	4.1–7.9	Eligible
Group 2 (1 : 0.9)	6.09±0.13	5.47±0.07	4.1–7.9	Eligible
Group 3 (1 : 0.8)	5.54±0.17	5.97±0.07	4.1–7.9	Eligible
Group 4 (1 : 0.7)	6.40±0.17	6.53±0.60	4.1–7.9	Eligible

Turbidity test

Observations on the turbidity test were carried out using visual observation to compare the level of turbidity of each dosage formulation over time. During this observation, the formulation preparations were placed in their respective containers and left to stand for 14 days. Observations were made on day 1, day 7, and day 14.

On the first day of observation, each dosage formulation was light yellow with clear turbidity. On the 7th day of observation, each formulation began to appear cloudy. Furthermore, on the 14th day, all preparations showed a light yellow color, but there was an increase in turbidity, as seen in Table 4.

Table 4. Turbidity test.

Group (ratio chitosan : NaTPP)	Day 1		7		14	
	Color	Turbidity	Color	Turbidity	Color	Turbidity
Group 1 (1 : 1.0)	Yellow	Clear	Yellow	Cloudy	Yellow	Cloudy
Group 2 (1 : 0.9)	Yellow	Clear	Yellow	Cloudy	Yellow	Cloudy
Group 3 (1 : 0.8)	Yellow	Clear	Yellow	Cloudy	Yellow	Cloudy
Group 4 (1 : 0.7)	Yellow	Clear	Yellow	Cloudy	Yellow	Cloudy

Viscosity test

Normality test results of the viscosity of chitosan and NaTPP encapsulation in entrapping kitchen lemongrass leaf extract in a 1:1, 1:0.9, 1:0.8, and 1:0.7 ratio shows a p value <0.05. Statistical testing was carried out non-parametrically using Friedman which showed a p value = 0.42, The viscosity test results did not show any differences between the encapsulation groups, were observed on the first, seventh, and 14th days.

The average results of the viscosity test on the first day between chitosan encapsulation and NaTPP with a ratio of 1:1 and 1:0.7 showed a significant difference with a p value = 0.04. Still, the 1:1 comparison group was not significantly different from the 1:0.8 and 1:0.9 comparison group. For days 7 and 14, there were no significant differences in all groups of the study. The average results of viscosity observations can be seen in Table 5.

On observation from the first day to day 14, the viscosity value of the chitosan encapsulation solution and NaTPP of lemongrass leaf extract 1:1 was relatively stable. The viscosity of the chitosan encapsulation solution and NaTPP of lemongrass leaf extract was 1:0.9 from the beginning of the observation until the 14th day, showing quite good stability. Encapsulation with a ratio of chitosan and NaTPP of lemongrass leaf extract of 1:0.8 showed that the viscosity value from the beginning of the observation until the 14th day saw a decrease in viscosity. Observation of the viscosity value at a ratio of 1:0.7 shows an increase in viscosity from the beginning of the observation to the 14th day. The results of viscosity testing in all comparison groups of chitosan and NaTPP show viscosity values within the standard mouthwash viscosity< 7.25.

Table 5. Viscosity test.

Group (ratio chitosan : NaTPP)	Viscosity level (mPa s)			Viscosity [12]	Description
	Day 1	Day 7	Day 14		
Group 1 (1 : 1.0)	2.169±0.04	2.138±0.01	2.134±0.01	< 7.25	Eligible
Group 2 (1 : 0.9)	2.106±0.01	2.118±0.01	2.118±0.01	< 7.25	Eligible
Group 3 (1 : 0.8)	2.132±0.05	2.120±0.02	2.116±0.02	< 7.25	Eligible
Group 4 (1 : 0.7)	2.081±0.02	2.127±0.01	2.165±0.11	< 7.25	Eligible

The research results showed that all comparison groups of chitosan and NaTPP encapsulation met the viscosity requirements for a mouthwash. A 1:1 ratio of chitosan and NaTPP had the best encapsulation physical characteristics with an even particle size distribution (monodisperse), so it met the requirements as a drug particle in the pharmaceutical field.

DISCUSSION

Encapsulation is a method used to package a compound within a material wall called a coating. This method offers advantages such as the stability of an active ingredient and increasing the safety of the material; as a result, the release of the compound is easier [13]. Encapsulation can be contained in several forms, such as capsules, emulsions, and particles [14]-[18].

This research used chitosan encapsulation to coat the active compounds of lemongrass leaves obtained from the extraction process using 70% ethanol solvent. An ethanol solvent concentration of 70% showed better antioxidant activity through the DPPH test compared to 50% and 90% ethanol solvent concentrations [19],[20]. However, research by Adiningsih *et al.* showed that compounds with 70% ethanol extract had antibacterial activity that was not much different with compounds extracted using 96% ethanol [21],[22]. This difference can occur because the 70% solvent is able to extract a large number of antioxidant compounds, such as flavonoids and phenolics, from plants. This result is proven by research by Nichitoi et al., which states that 70% ethanol extract is able to attract more compounds, but 50% ethanol extract has better antibacterial activity [23]. In addition, research by Rahayu *et al.* states that 96% ethanol extract of roots *Zingiber zerumbet* has the best antibacterial activity against *S. enteriditis* and *S. typhimurium* bacteria compared with concentrations of 45% and 70% [24].

The results of particle measurements in encapsulated preparations showed that the particle size in preparations with a chitosan and NaTPP ratio of 1:1 reached 524.0 nm, smaller than the other comparison groups, namely 1:0.9 (614.5 nm), 1:0.8 (934.2 nm), and 1:0.7 (1145 nm). A particle is said to be nano-sized if the particle has a size between 1 and 1000 nm [6,16]. In addition, of the four preparation ratios, the ratio of chitosan and NaTPP is 1:1, 1:0.9, and 1:0.8 can be classified in the nano size category, while compounds with a ratio of 1:0.7 do not meet the requirements as nanoparticles. In Khoerunnisa's research, the 1:1 ratio variation is a synthesis with optimum characteristics. Variations in the ratio of chitosan: NaTPP are different from research conducted by Dwiki et al., which reported an increase in size with each increase in the ratio of chitosan: NaTPP for bay leaves (*Syzygium polyanthum*), namely 1:1 (284 nm) and 5:1 (410 nm).) and 10:1 (630 nm). This results is different from research conducted by Rizal *et al.,* who reported that there was a parabolic relationship in variations in the chitosan-NaTPP composition of jackfruit leaves (*Artocarpus heterophyllus* Lam.), namely 1:1 (382.6 nm), 2:1 (199.2 nm), and 3:1 (402.2 nm). These results explain that variations and formulations of the chitosan : NaTPP ratio can provide significant differences in particle size [25].

Particle distribution testing can use the Polydispersity Index (PdI) approach. Particles with a PdI value <0.7 can be said to be monodisperse, and a PdI value > 0.7 indicates that they are polydisperse particles [26]. In addition, monodisperse particles can also be considered good because they are located in the middle of the polydispersity index range. The average value of the index PdI in encapsulation with a ratio of chitosan and NaTPP, namely 1:1, 1:0.9, and 1:0.8, shows a PdI figure <0.7, respectively 0.481, 0.695, and 0.687. This results shows that the three preparations have monodisperse character. Monodispersion of a nanoparticle means that there is uniformity in the size of the particles formed [27],[28]. This can provide benefits through a greater opportunity to interact with the biological target [29]. Furthermore, encapsulation preparations with a ratio of chitosan and NaTPP 1:0.7 have a value of 0.747, which indicates that the sample is polydisperse, that is, the comparative particles have parts with a wide particle size and have particles with a large size [28].

The presence of active compounds in the formulation preparation can be analyzed to assess the characteristics of the compound's functional groups [30]. In this case, to confirm that the chitosan nanoparticles do not decompose during the encapsulation preparation process, they can be analyzed using an FTIR approach. In the encapsulated preparation, there is an O-H absorption area, which looks steep and wide; this indicates a mixture of O-H from chitosan, NaTPP, and 70% ethanol extract of lemongrass leaves. From the observation process, it was seen that C=O absorption was in the area of 1638.60 – 1645.35 cm^{-1} for each formulation, where there was a shift from the area of 1627.99 cm-1 of pure chitosan extract. This shift indicates an interaction between chitosan and the 70% ethanol extract of lemongrass leaves. It is a sign that the chitosan compound is still available in each encapsulation formulation [8], [30]-[32]. Apart from that, there is also a shift in the bending N-H absorption area from 1579.77 cm^{-1} to a number area ranging between 1550.83–1566.27 cm^{-1} for each encapsulation formulation. This shift indicates that cross-linking has occurred between the chitosan compound, NaTPP, and the 70% ethanol extract of lemongrass leaves [8],[31],[32]. Furthermore, the

absorption peak of the P-O group was obtained in the area of 917.19 - 922.98 cm^{-1} for the encapsulation formulation, which indicates the P-O aliphatic group of NaTPP [30],[33].

When observing the turbidity level for 14 days, it was found that changes occurred in each dosage formulation [34]-[37]. On the 7th day, minimal changes occurred. This is slightly different from research conducted by Syarmalina *et al.*, which reported the stability of the turbidity level in the temu lawak extract nanoparticle formulation, which was observed for 5 days [38]. This change could occur due to several background factors, such as nanoparticle size, size distribution, and concentration [39].

For mouthwash, the viscosity component is necessary to review the comfort of the solution when consumed [33],[40]. This is because the closer the viscosity value of a formulation is to the viscosity of water (1 cP), the easier and more comfortable the formulation is to consume [33],[41]. The smaller the viscosity value, the more dilute the solution; conversely, the greater the viscosity value, the thicker the solution [42]. In this study, the viscosity value of the chitosan encapsulation solution and NaTPP leaf extract on day 14 ranged from 2.116 – 2.165 cP, the highest viscosity value was found by the formulation with a ratio of 1:0.7 and the lowest was owned by the formulation with a ratio of 1:0.8. Formulations with a ratio of 1:1 and 1:0.9 were relatively stable during the first to 14th day of observation. This result is different from the encapsulation formulation with a chitosan and NaTPP ratio of lemongrass leaf extract of 1:0.8, which shows a decrease in viscosity, and a formulation of 1:0.7, which shows an increase in viscosity. This viscosity instability can be caused by the inhomogeneity of the particles in the formulation [33]. Apart from that, the solution is easy to decompose by temperature and light. Generally the viscosity of the liquid will decrease with increasing temperature [34]. In addition, the viscosity values of the four formulations are still below standard (<7.25), so it still meets the standard mouthwash viscosity value requirements.

Thus, the encapsulation ratio 1:1 is the most optimal choice among other ratios. This preparation has a nanoparticle size with a uniform distribution (monodispersion), making it easier for this compound to interact with its biological target. In addition, the stability and good viscosity of this preparation also support these advantages, creating an optimal system for applications in the medical field. Overall, this preparation offers a potential solution for therapeutic development in the context of mouthwash.

▪ CONCLUSION

The physical characteristics of the encapsulation of kitchen lemongrass leaf extract for mouthwash with several comparisons of chitosan and NaTPP have good particle size, pH value, turbidity, and viscosity, as well as cross-linking between the chitosan compound, NaTPP, and 70% ethanol extract of lemongrass leaves, which was confirmed through the FT-IR test.

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Characteristic and therapeutic effect of lemongrass leaf encapsulation with a different ratio of chitosan and NaTPP

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Characteristic and therapeutic effect of lemongrass leaf encapsulation with a different ratio of chitosan and NaTPP

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ABSTRACT: The lemongrass plant (*Cymbopogon citratus*) in Indonesia has great potential as a natural ingredient for medicines because it contains various compounds such as neral, citral, geranial acetate, flavonoids, and tannins. Previous research has demonstrated various pharmacological activities of kitchen lemongrass leaves, including antibacterial, antifungal, and anti-inflammatory properties. Encapsulation technology in the form of chitosan-NaTPP nanoparticles is used to maintain the activity of kitchen lemongrass leaf compounds. This research aims to determine the physical characteristics of the encapsulation of kitchen lemongrass leaf extract with several comparisons of chitosan and NaTPP by testing PSA (particle size analysis test), FTIR (functional group analysis test), viscosity, and stability, including pH and turbidity tests. Encapsulation with a 1:1 ratio of chitosan and NaTPP has a particle size of 524 nm and a PdI value of 0.481, classified as monodisperse (PdI <0.7). The pH value ranges from 6.46 to 7.30, and the viscosity value was 2.134 – 2.169 cP, which is still within mouthwash standards. The turbidity test showed stable encapsulation results. Therefore, encapsulation with a 1:1 ratio of chitosan and NaTPP is the most optimal choice and has potential for developing the therapeutic effect of mouthwash.

KEYWORDS: Chitosan; encapsulation; lemongrass leaves; mouthwash; NaTPP

• INTRODUCTION

Indonesia is rich in natural plant sources as raw materials for medicines. One plant that is widely used is the kitchen lemongrass leaves. Many researchers are taking advantage of technological advances and making various efforts to improve the quality and trust in the benefits of natural sources, one of which is kitchen lemongrass leaves [1]. *Cymbopogon citratus*, commonly called kitchen lemongrass, has a larger lower stem, is white, and does not smell too sharp. The part that is often used as a kitchen spice is the young inner part. Lemongrass plays a role as an aromatic spice in cooking and is also used as a complement to chili sauce. These plants can both produce essential oils, but in Indonesia, only citronella is widely processed as an essential oil producer [2]. The results of various studies show that citronella leaves are rich in essential oils and contain neral (31.55%), citral (26.1%), and geranial acetate (2.27%). This ingredient is often used as an aromatherapy oil [3]. This plant also contains active compounds, namely flavonoids, saponins, tannins, phenols, aldehydes, phenolic acids, and terpenoids. Studies also show that lemongrass leaves have various pharmacological properties, such as antibacterial, antifungal, and anti-inflammatory, especially when used as a mouthwash [4].

One way that can be used to protect secondary metabolic activity in lemongrass leaf extract is through encapsulation technology, which is a technique for covering secondary metabolism to protect it from external influences. Encapsulation is divided into micro and nanoparticle forms. Applications of food nanotechnology show an increasing trend. This technology offers advantages in increasing secondary metabolic bioavailability, controlling the release of active compounds, and improving sensory properties [5]. Nanoparticles are solid colloidal particles with a diameter of 1-1000 nm and are materials that can be used as encapsulated active compounds [6]. The active compound of lemongrass leaves has poor stability, so it is encapsulated with horn beetle chitosan to increase its stability, such as antioxidant, anti-cancer, anti-inflammatory, anti-fungal, and antibacterial [7].

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Chitosan is a natural polysaccharide that is non-toxic and easily biodegradable in delivery systems because it has properties as a matrix. Chitosan also forms gels in acidic conditions and has a structure similar to chitin [8]. Chitosan is a compound of poly (N-amino-2-deoxy-β-D-glucopyranose) or glucosamine resulting from the deacetylation of chitin/poly (N-acetyl2-amino-2-deoxy-βDglucopyranose) [9].

Previous studies by Purwandari showed that moringa leaf extract was obtained by repeated ultrasonication of 1x15, 3x15, and 5x15 minutes, frequency 24 kHz, and using 96% alcohol solvent [9]. The encapsulation process was carried out with three variable ratios of the amount of chitosan, Na-TPP added to 10 mL of moringa leaf extract solution, namely 5:1, 2:1 and 1:1. The results of the flavonoid test as quercetin using a UV-Vis spectrophotometer showed that the quercetin content in moringa leaf extract resulting from 5x15 minute ultrasonication was 12.146%, Particle Size Analyzer (PSA) characterization showed encapsulated particle sizes of 170.3 and 209.7 nm with a polydispersion index <0.5, nanoencapsulation of moringa leaf extract has been produced. There has been an increase in the antioxidant activity of moringa leaf extract after encapsulation using chitosan-NaTPP 2:1, namely from 61.4% to 81.2% [9].

Based on the background above, this research was conducted to determine the physical characteristics of chitosan encapsulation of kitchen lemongrass leaf extract with several comparisons of chitosan and NaTPP, which will determine the activity of the encapsulated particles as antibacterial, anticancer, and antioxidant.

MATERIALS AND METHODS

Materials

This study was conducted with lemongrass leaf extract (Bogor, Indonesia), 70% ethanol, 1% acetic acid, distilled water, chitosan, NaTPP, and Tween 80.

Kitchen lemongrass leaf extraction

Lemongrass leaf simplicia was extracted using the maceration method by soaking samples of kitchen lemongrass leaves in 70% ethanol with a ratio of 1:10 for 3 × 24 hours at a temperature of 27 °C. The solution will be filtered using Whatman filter paper and spun using an evaporator machine at 50-60 °C.

Encapsulation of lemongrass leaf extract

The nano chitosan encapsulation stage was achieved by dissolving 40 mL of chitosan in 1% acetic acid and adding 100 mL of distilled water. Lemongrass leaf extract is dissolved using distilled water. Then, the extract solution was mixed into the chitosan solution. Stirring was carried out at a speed of 2500 rpm for 2 hours. Next, tripolyphosphate was given drop by drop. Finally, 0.1 mL of Tween 80 was added to the solution. In making the encapsulation, a comparison was made between the extract and chitosan, presented in **Table 1.**

Table 1. Encapsulation comparison.

Group (ratio chitosan : NaTPP)	Chitosan (mg/mL)	NaTPP (mL)	Lemongrass (mL)	Tween 80 (mL)
Group 1 (1 : 1.0)	0.57	0.57	2	0.1
Group 2 (1 : 0.9)	0.57	0.514	2	0.1
Group 3 (1 : 0.8)	0.57	0.468	2	0.1
Group 4 (1 : 0.7)	0.57	0.429	2	0.1

Particle size analysis test

An encapsulated sample of lemongrass leaf extract in liquid form was prepared and dropped into a cuvette to which sufficient distilled water was added as a solvent. Then, it was put into the PSA apparatus, and the temperature was set to 25 °C. The results of the sample particle size distribution will appear on the monitor.

FTIR test

The FTIR (Fourier Transform Infrared Spectroscopy) test was used to analyze the functional groups contained in the sample. The chemical interactions between NaTPP, chitosan, and kitchen lemongrass leaf extract were analyzed using an FTIR spectrophotometer at wave numbers 4000 – 400 cm^{-1}. Analysis was carried out using the Shimadzu Prestige-21 FTIR tool. A total of 1 mL of encapsulated sample for each formulation was frozen using a freeze dryer until it reached a temperature of -80 °C. Then, the sample was put into the tool and analyzed. Next, the results are displayed on the monitor.

Stability test

Turbidity test

Turbidity test by visible observation. This observation aims to see the occurrence of material aggregation within 2 weeks. The measurement results were color and turbidity.

pH determination test

Acid measurements were carried out using a Mettler Toledo pH meter. First, prepare the sample whose acid level you want to measure (place it in a container). Next, the electrode from the pH meter was inserted into a container containing the solution to be tested. When immersed in the solution, the number scale will move randomly. Wait until the number stops and does not change. The results will be visible on the monitor.

Viscosity test

The viscosity test was carried out through measurements using the ViscoQC 100 tool Anton Paar (rotational viscometer). The time is set to 1 minute, the torque is 56%, and the speed is 200 rpm. The results displayed on the monitor screen are recorded. Viscosity measurements were carried out on days 1, 7, and 14.

RESULTS

Particle size analysis test (PSA)

The PSA measurements showed that the encapsulation group with a 1:1 ratio of chitosan and NaTPP had the smallest size of 524.0 nm compared to the other comparison groups. The comparison group for chitosan and NaTPP encapsulation, which has the largest to smallest size, was the ratio of chitosan and NaTPP encapsulation 1:0.7, 1:0.8, 1:0.9, and 1:1, with particle sizes of 1145 nm, 934.2 nm, 614.5 nm, and 524 nm, respectively, as shown in **Table 2.** Particle measurement results, the ratio of chitosan and NaTPP encapsulation was 1:0.8, 1:0.9, and 1:1, which fall into nano-sized particles, while the encapsulation ratio of chitosan and NaTPP 1:0.7 with a size of more than 1000 nm does not meet the requirements for particle preparation.

Polydispersity Index (PdI) is an index of the heterogeneity of a collection of particles. The average PdI value of the encapsulation ratio of chitosan and NaTPP was 1:1, 1:0.9, and 1:0.8, with PdI values of 0.481, 0.695, and 0.687, respectively. PdI values from a 1:1 chitosan encapsulation ratio of 1:0.9 and 1:0.8 have a PdI value of <0.7. According to Chasana et al., particles can be classified into monodisperse groups (PdI value <0.7) and polydisperse large size distribution (PdI value>0.7). A PdI value <0.7 indicates good particles because it is in the middle range of the polydispersity index. For encapsulation, the ratio of chitosan and NaTPP 1:0.7 has a value of 0.747, which indicates that the sample has a part with a large particle size and is not homogeneous.

Table 2. Particle size and polydispersity index.

Group (ratio chitosan : NaTPP)	Particle size (nm)	PdI
Group 1 (1 : 1.0)	524.0	0.481
Group 2 (1 : 0.9)	614.5	0.695
Group 3 (1 : 0.8)	934.2	0.687
Group 4 (1 : 0.7)	1145	0.747

FTIR test

The graphical image of the analysis of the FTIR test results showed that several functional groups were identified in the four samples (**Figure 1**). In the chitosan extract sample, there were absorption peaks in the wave number area 3425 cm^{-1} (O-H stretching vibrations overlap with N-H), 1627.99 cm^{-1} (C=O Amide I stretching vibrations), 1579.77 cm^{-1} (C=O Amide I stretching vibrations). NH_2 bending in Amide II). The four

chitosan encapsulation preparations experienced a slight shift in the absorption peak at the amide I carbonyl group, namely around the number area 1642.46 cm^{-1} for the 1:1 formulation, 1640.53 cm^{-1} for the 1:0.9 formulation, 1638.60 cm^{-1} for the 1:0.8 formulation, and 1645.35 cm^{-1} for the 1:0.7 formulation. Furthermore, there is also a shift in N-H bending vibrations in the area of 1566.27 cm^{-1} for the 1:1 formulation, 1564.34 cm^{-1} for the 1:0.9 formulation, 1550.83 cm^{-1} for the 1:0 formulation 8, and 1563.37 cm^{-1} for the 1:0.7 formulation. Besides that, there was an absorption peak of the P-O group in the number area 917.19 cm^{-1} for the 1:1 formulation, 919.12 cm^{-1} for the 1:0.9 formulation, and 921.05 cm^{-1} for the 1:0.8 formulation. Next, the formulation 1:0.7 was located in the number area 922.98 cm^{-1}.

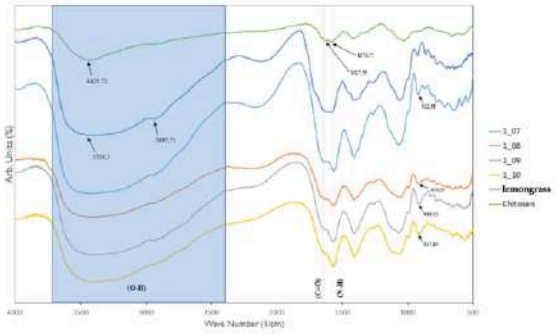

Figure 1. FTIR analysis results.

pH determination

The results of the pH normality test of encapsulated chitosan and NaTPP in trapping lemongrass leaf extract in a ratio of 1:1, 1:0.9, 1:0.8, and 1:0.7, with p value>0.05. The normality test results were followed by a repeated ANOVA test to see whether there was a difference in pH values on the first day and the seventh day. The parametric test results showed no significant difference in pH values on the first and seventh days ($p>0.05$). The pH test results can be seen in **Table 3**.

Encapsulation of chitosan and NaTPP in entrapping lemongrass leaf extract in a ratio of 1:1; 1:0.9; 1:0.8; and 1:0.7 on the first day of pH observation showed that the encapsulation group with a ratio of 1:0.8 was significantly different ($p<0.05$) from the other encapsulation groups, which were shown by the lowest pH value, namely 5.54 ±0.17. On the seventh day, the pH of the 1:1 ratio encapsulation group was not significantly different from the 1:0.7 ratio with a p value of 0.06, but was significantly different from the 1:0.8 and 1:0.9 ratio encapsulation groups ($p<0.05$). Each formulation has an increase and a decrease in the pH value. Encapsulation 1:1, 1:0.8, and 1:0.7 experienced an increase in pH on the seventh day. Meanwhile, encapsulation 1:0.9 saw a decrease in pH on the seventh day. Changes in pH values can be observed in **Table 3**.

Table 3. pH determination test.

Group (ratio chitosan : NaTPP)	pH level		pH of commercial mouthwash [35]	Description
	Day 1	Day 7		
Group 1 (1 : 1.0)	6.46±0.14	7.30±0.14	4.1 – 7.9	E
Group 2 (1 : 0.9)	6.09±0.13	5.47±0.07	4.1 – 7.9	E
Group 3 (1 : 0.8)	5.54±0.17	5.97±0.07	4.1 – 7.9	E
Group 4 (1 : 0.7)	6.40±0.17	6.53±0.60	4.1 – 7.9	E

Description:

E : Eligible
NE: Not Eligible

Turbidity test

Observations on the turbidity test were carried out using visual observation to compare the level of turbidity of each dosage formulation over time. During this observation, the formulation preparations were placed in their respective containers and left to stand for 14 days. Observations were made on day 1, day 7, and day 14.

On the first day of observation, each dosage formulation was light yellow with clear turbidity. On the 7th day of observation, each formulation began to appear cloudy. Furthermore, on the 14th day, all preparations showed a light yellow color, but there was an increase in turbidity, as seen in **Table 4**.

Table 4. Turbidity test.

Group (ratio chitosan : NaTPP)	Day					
	1		7		14	
	Color	Turbidity	Color	Turbidity	Color	Turbidity
Group 1 (1 : 1.0)	Yellow	Clear	Yellow	Cloudy	Yellow	Cloudy
Group 2 (1 : 0.9)	Yellow	Clear	Yellow	Cloudy	Yellow	Cloudy
Group 3 (1 : 0.8)	Yellow	Clear	Yellow	Cloudy	Yellow	Cloudy
Group 4 (1 : 0.7)	Yellow	Clear	Yellow	Cloudy	Yellow	Cloudy

Viscosity test

Normality test results of the viscosity of chitosan and NaTPP encapsulation in entrapping kitchen lemongrass leaf extract in a 1:1, 1:0.9, 1:0.8, and 1:0.7 ratio shows a p value <0.05. Statistical testing was carried out non-parametrically using Friedman which showed a p value = 0.42, The viscosity test results did not show any differences between the encapsulation groups, were observed on the first, seventh, and 14th days.

The average results of the viscosity test on the first day between chitosan encapsulation and NaTPP with a ratio of 1:1 and 1:0.7 showed a significant difference with a p value = 0.04. Still, the 1:1 comparison group was not significantly different from the 1:0.8 and 1:0.9 comparison group. For days 7 and 14, there were no significant differences in all groups of the study. The average results of viscosity observations can be seen in **Table 5**.

On observation from the first day to day 14, the viscosity value of the chitosan encapsulation solution and NaTPP of lemongrass leaf extract 1:1 was relatively stable. The viscosity of the chitosan encapsulation solution and NaTPP of lemongrass leaf extract was 1:0.9 from the beginning of the observation until the 14th day, showing quite good stability. Encapsulation with a ratio of chitosan and NaTPP of lemongrass leaf extract of 1:0.8 showed that the viscosity value from the beginning of the observation until the 14th day saw a decrease in viscosity. Observation of the viscosity value at a ratio of 1:0.7 shows an increase in viscosity from the beginning of the observation to the 14th day. The results of viscosity testing in all comparison groups of chitosan and NaTPP show viscosity values within the standard mouthwash viscosity< 7.25.

Table 5. Viscosity test.

Group (ratio chitosan : NaTPP)	Viscosity level (mPa s)			Viscosity (Depkes RI. 2020)	Description
	Day 1	Day 7	Day 14		
Group 1 (1 : 1.0)	2.169±0.04	2.138±0.01	2.134±0.01	< 7.25	E
Group 2 (1 : 0.9)	2.106±0.01	2.118±0.01	2.118±0.01	< 7.25	E
Group 3 (1 : 0.8)	2.132±0.05	2.120±0.02	2.116±0.02	< 7.25	E
Group 4 (1 : 0.7)	2.081±0.02	2.127±0.01	2.165±0.11	< 7.25	E

Description :
E : Eligible
NE: Not Eligible

The research results showed that all comparison groups of chitosan and NaTPP encapsulation met the viscosity requirements for a mouthwash. A 1:1 ratio of chitosan and NaTPP had the best encapsulation

physical characteristics with an even particle size distribution (monodisperse), so it met the requirements as a drug particle in the pharmaceutical field.

DISCUSSION

Encapsulation is a method used to package a compound within a material wall called a coating [11]. This method offers advantages such as the stability of an active ingredient and increasing the safety of the material; as a result, the release of the compound is easier [12,13]. Encapsulation can be contained in several forms, such as capsules, emulsions, and particles [14-18].

This research used chitosan encapsulation to coat the active compounds of lemongrass leaves obtained from the extraction process using 70% ethanol solvent. An ethanol solvent concentration of 70% showed better antioxidant activity through the DPPH test compared to 50% and 90% ethanol solvent concentrations[19,20]. However, research by Adiningsih *et al.* showed that compounds with 70% ethanol extract had antibacterial activity that was not much different with compounds extracted using 96% ethanol [21]. This difference can occur because the 70% solvent is able to extract a large number of antioxidant compounds, such as flavonoids and phenolics, from plants. This result is proven by research by Nichitoi et al., which states that 70% ethanol extract is able to attract more compounds, but 50% ethanol extract has better antibacterial activity [23]. In addition, research by Rahayu *et al.* states that 96% ethanol extract of roots *Zingiber zerumbet* has the best antibacterial activity against *S. enteriditis* and *S. typhimurium* bacteria compared with concentrations of 45% and 70% [24].

The results of particle measurements in encapsulated preparations showed that the particle size in preparations with a chitosan and NaTPP ratio of 1:1 reached 524.0 nm, smaller than the other comparison groups, namely 1:0.9 (614.5 nm), 1:0.8 (934.2 nm), and 1:0.7 (1145 nm). A particle is said to be nano-sized if the particle has a size between 1 and 1000 nm [6,16]. In addition, of the four preparation ratios, the ratio of chitosan and NaTPP is 1:1, 1:0.9, and 1:0.8 can be classified in the nano size category, while compounds with a ratio of 1:0.7 do not meet the requirements as nanoparticles. In Khoerunnisa's research, the 1:1 ratio variation is a synthesis with optimum characteristics. Variations in the ratio of chitosan: NaTPP are different from research conducted by Dwiki et al., which reported an increase in size with each increase in the ratio of chitosan: NaTPP for bay leaves (*Syzygium polyanthum*), namely 1:1 (284 nm) and 5:1 (410 nm).) and 10:1 (630 nm). This results is different from research conducted by Rizal *et al.*, who reported that there was a parabolic relationship in variations in the chitosan-NaTPP composition of jackfruit leaves (*Artocarpus heterophyllus* Lam.), namely 1:1 (382.6 nm), 2:1 (199.2 nm), and 3:1 (402.2 nm). These results explain that variations and formulations of the chitosan : NaTPP ratio can provide significant differences in particle size [25].

Particle distribution testing can use the Polydispersity Index (PdI) approach. Particles with a PdI value < 0.7 can be said to be monodisperse, and a PdI value > 0.7 indicates that they are polydisperse particles [26]. In addition, monodisperse particles can also be considered good because they are located in the middle of the polydispersity index range. The average value of the index PdI in encapsulation with a ratio of chitosan and NaTPP, namely 1:1, 1:0.9, and 1:0.8, shows a PdI figure <0.7, respectively 0.481, 0.695, and 0.687. This results shows that the three preparations have monodisperse character. Monodispersion of a nanoparticle means that there is uniformity in the size of the particles formed [27,28]. This can provide benefits through a greater opportunity to interact with the biological target.[29] Furthermore, encapsulation preparations with a ratio of chitosan and NaTPP 1:0.7 have a value of 0.747, which indicates that the sample is polydisperse, that is, the comparative particles have parts with a wide particle size and have particles with a large size [28].

The presence of active compounds in the formulation preparation can be analyzed to assess the characteristics of the compound's functional groups [30]. In this case, to confirm that the chitosan nanoparticles do not decompose during the encapsulation preparation process, they can be analyzed using an FTIR approach. In the encapsulated preparation, there is an O-H absorption area, which looks steep and wide; this indicates a mixture of O-H from chitosan, NaTPP, and 70% ethanol extract of lemongrass leaves. From the observation process, it was seen that C=O absorption was in the area of 1638.60 – 1645.35 cm^{-1} for each formulation, where there was a shift from the area of 1627.99 cm-1 of pure chitosan extract. This shift indicates an interaction between chitosan and the 70% ethanol extract of lemongrass leaves. It is a sign that the chitosan compound is still available in each encapsulation formulation.[8,30-32] Apart from that, there is also a shift in the

bending N-H absorption area from 1579.77 cm^{-1} to a number area ranging between 1550.83–1566.27 cm^{-1} for each encapsulation formulation. This shift indicates that cross-linking has occurred between the chitosan compound, NaTPP, and the 70% ethanol extract of lemongrass leaves. [8,31,32] Furthermore, the absorption peak of the P-O group was obtained in the area of 917.19 - 922.98 cm^{-1} for the encapsulation formulation, which indicates the P-O aliphatic group of NaTPP [30,33].

When observing the turbidity level for 14 days, it was found that changes occurred in each dosage formulation. On the 7th day, minimal changes occurred. This is slightly different from research conducted by Syarmalina *et al.*, which reported the stability of the turbidity level in the temu lawak extract nanoparticle formulation, which was observed for 5 days [38]. This change could occur due to several background factors, such as nanoparticle size, size distribution, and concentration [39].

For mouthwash, the viscosity component is necessary to review the comfort of the solution when consumed.[33,40] This is because the closer the viscosity value of a formulation is to the viscosity of water (1 cP), the easier and more comfortable the formulation is to consume [33,41]. The smaller the viscosity value, the more dilute the solution; conversely, the greater the viscosity value, the thicker the solution [42]. In this study, the viscosity value of the chitosan encapsulation solution and NaTPP leaf extract on day 14 ranged from 2.116 – 2.165 cP, the highest viscosity value was found by the formulation with a ratio of 1:0.7 and the lowest was owned by the formulation with a ratio of 1:0.8. Formulations with a ratio of 1:1 and 1:0.9 were relatively stable during the first to 14th day of observation. This result is different from the encapsulation formulation with a chitosan and NaTPP ratio of lemongrass leaf extract of 1:0.8, which shows a decrease in viscosity, and a formulation of 1:0.7, which shows an increase in viscosity. This viscosity instability can be caused by the inhomogeneity of the particles in the formulation [33]. Apart from that, the solution is easy to decompose by temperature and light. Generally the viscosity of the liquid will decrease with increasing temperature [34]. In addition, the viscosity values of the four formulations are still below standard (< 7.25), so it still meets the standard mouthwash viscosity value requirements.

Thus, the encapsulation ratio 1:1 is the most optimal choice among other ratios. This preparation has a nanoparticle size with a uniform distribution (monodispersion), making it easier for this compound to interact with its biological target. In addition, the stability and good viscosity of this preparation also support these advantages, creating an optimal system for applications in the medical field. Overall, this preparation offers a potential solution for therapeutic development in the context of mouthwash.

CONCLUSION

The physical characteristics of the encapsulation of kitchen lemongrass leaf extract for mouthwash with several comparisons of chitosan and NaTPP have good particle size, pH value, turbidity, and viscosity, as well as cross-linking between the chitosan compound, NaTPP, and 70% ethanol extract of lemongrass leaves, which was confirmed through the FT-IR test.

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