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Synthesis and Characterization of Ethanol Extract Nanoherb of Tapak Liman Leaves (Elephantopus scaber Linn.) by Ionic Gelation Method

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Abstract— Tapak Liman (Elephantopus scaber) is a plant that has been used by Indonesian people as a traditional medicine to treat diarrhea, fever, malaria, and coughs. Tapak Liman leaves contain compounds of flavonoids, phenols, and saponins. However, these phytochemical compounds exhibit low bioavailability properties. Therefore, a technology that can improve their bioavailability is needed. One approach is through the synthesis of Tapak Liman nanoparticles via the ionic gelation method using alginate and $CaCl_2$. This study aims to determine the content of Tapak Liman leaves, synthesize nanoherb from its ethanol extract using alginate and $CaCl_2$, and determine the nanoherb's particle size and zeta potential. Nanoherbs were synthesized through the ionic gelation method using alginate and $CaCl_2$ with concentrations of sodium alginate at 0.15% w/v; 0.10% w/v; and 0.05% w/v; while the concentration of $CaCl_2$ was at 0.01% w/v. Particle size characterization was performed on all three formulations to identify the optimal one for further zeta potential characterization. The results showed that Tapak Liman leaves contain saponins, triterpenoids, tannins, and phenolics. The optimal formulation for synthesizing ethanol extract nanoherb of Tapak Liman leaves is sodium alginate 0.05% w/v and $CaCl_2$ 0.01% w/v, resulting in a nanoherb with a particle size of 184.7 nm, a volume percentage of 51.1%, a polydispersity index of 0.370, and a zeta potential of 19.2 mV. This indicates the potential of Tapak Liman leaf ethanol extract nanoherbs in drug delivery systems, contingent upon stability enhancement.

Keywords— alginate; $CaCl_2$; characterization; tapak liman leaves; nanoherb

I. INTRODUCTION

Tapak Liman (*Elephantopus scaber* Linn.) is a plant that has been used by many as a traditional medicine to treat diarrhea, fever, malaria, and coughs [1]. Traditionally, Tapak Liman is known for its bioactive properties, including antipyretic, antihepatotoxic, and anti-inflammatory properties [2]. Additionally, Tapak Liman acts as an antibacterial agent, which can inhibit bacterial growth through its flavonoid, phenolic, and saponin components [3], [4]. These three compounds can be extracted with ethanol, a universal, polar, and readily available solvent. However, these phytochemical compounds contained in Tapak Liman leaves exhibit low bioavailability properties [5], [6], [7]. The low bioavailability of herbal active compounds in the form of extracts is due to its low water solubility [8]. Therefore, a technology that can improve its bioavailability is needed. One way to accomplish this is through the synthesis of Tapak Liman's nanoparticles [9].

Nanotechnology is a field which aims to develop drug particles within the size range of 1–1000 nm [10]. Nanotechnology offers several benefits, including modifiable surface characteristics and particle sizes, as well as the ability to deliver drugs without a high dosage. This supplements the effectiveness of a drug by increasing its selectivity due to its ability to target specific organs, and also increases safety due to reduced risk of overdose and side effects if it were delivered to an unintended organ. [11], [12].

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One nanotechnological approach to synthesize nanoparticles that utilizes herbal medicines using nanoparticle methods is called herbal nanoparticle, or nanoherb. And one of the methods used to synthesize nanoherb is the ionic gelation method [13].

The ionic gelation method is one of the nanoherb synthesis methods that utilizes ionic interactions between polyelectrolyte compounds and their multivalent ion pairs. This method offers advantages such as the use of safe materials, ease of application, and easily modifiable particle size [14]. Nanoherbs synthesized using this method have been carried out on several plant extracts, including Katuk leaves [15], Temu Kunci [16], and Kembang Sepatu leaves [17], all of which, similar to Tapak Liman, contain flavonoid, phenolic, and saponin compounds. However, studies regarding the synthesis of nanoherb of Tapak Liman leaves through the ionic gelation method have not yet been reported, presenting an opportunity to study this in further detail. The purpose of this study is to synthesize ethanol extract nanoherb of Tapak Liman leaves using the ionic gelation method and determine its characteristics.

II. Materials and Methods

2.1. Materials and Equipment

The equipment used in this study included a set of maceration tools, Buchner funnel (Haldenwanger), vacuum pump (Value VE225 N), filter paper (Whatman No. 42), Zetasizer Nano ZS (Malvern), vacuum rotary evaporator (Buchi R-300), magnetic stirrer (Thermo Scientific), analytical scales (Ohaus Adventurer AR2140), graduated cylinder (Pyrex), beaker glass (Pyrex), Erlenmeyer flask (Pyrex), petri dishes (Pyrex), pipette, and spatula. The materials used in this study included ground dried Tapak Liman leaves, sodium alginate (Merck), calcium chloride ($CaCl_2$) (Merck), 96% ethanol, ethanol p.a. (Merck), and aquadest.

2.2. Tapak Liman Leaves Extraction

Tapak Liman leaves were extracted by maceration with ethanol. Through the maceration extraction method, a 500 gram sample of Tapak Liman leaves was soaked in 2 liters of ethanol 96% for 24 hours in a closed container. The sample was then filtered with a Buchner funnel and a vacuum pump to obtain both the filtrate and the residue. The obtained filtrate was evaporated using a vacuum rotary evaporator at 30°C to obtain a concentrated extract. The extract was stored in a refrigerator at 4°C until use [18].

2.3. Nanoherbal Synthesis

To begin the nanoherb synthesis process, 1 gram of concentrated Tapak Liman leaves extract was put into a beaker glass. The extract was then dissolved in 35 mL of ethanol p.a. 15 mL of aquadest were then also added into the beaker glass to obtain a concentration of 2% w/v of the Tapak Liman leaves ethanol extract. 100 mL of sodium alginate solution and 350 mL of calcium chloride solution, at concentrations specified for each formula (Table I), were then gradually poured into the beaker glass containing the extract while being stirred with a magnetic stirrer at 1000 rpm for 2 hours. The nanoherb colloid solution was then centrifuged at 6000 rpm for 15 minutes to obtain its precipitate [19]. The precipitate was then dried using a freeze dryer for 8 hours to obtain a dry powder. The colloid solution is used in the particle size and zeta potential analysis, while the dry powder is used in the functional groups analysis by FTIR [20].

TABLE I. NANOHERB FORMULAS

Concentration (% w/v)	**Formula**		
	1	***2***	***3***
Ethanol extract of Tapak Liman leaves	2%	2%	2%
Sodium alginate	0.15%	0.1%	0.05%
Calcium chloride	0.01%	0.01%	0.01%

III. Result and Discussion

3.1. Tapak Liman Leaves Extraction

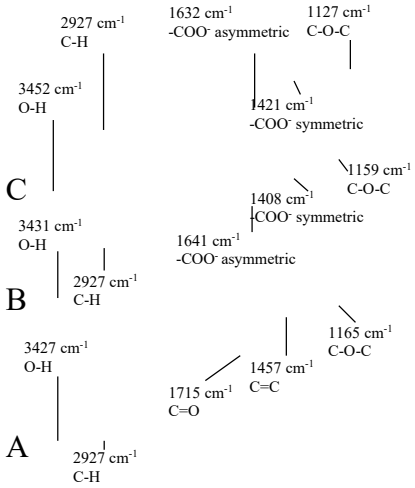
The extraction of Tapak Liman leaves was conducted via maceration. The maceration method was chosen because it does not use heat, to ensure that the secondary metabolites in the Tapak Liman leaves will not be damaged. Additionally, the maceration method offers the advantage of having an easily applicable process with simple equipment [21]. In the maceration process, ethanol was used as a solvent due to its universal, polar, and readily available properties. Ethanol with a concentration of 96% was chosen because it has the properties of being selective, non-toxic, and absorbing well compared to ethanol with lower concentrations [22]. In maceration, a significant amount of secondary metabolites may remain after the first process; therefore, a repeated maceration process was required. The filtrates were then mixed to combine the secondary metabolites contained in each filtrate [23]. The combined filtrates of the macerated extract were evaporated using a rotary evaporator to obtain a concentrated ethanol extract of Tapak Liman leaves. A temperature of 30°C was chosen because, although high temperatures can accelerate the extraction process by increasing the diffusion coefficient and the solute's solubility, temperatures above 50°C can damage the stability of the compounds inside the extract and cause membrane denaturation. Thus, the optimal temperature for extract evaporation is 30°C [24]. Through the evaporation, a thick and dark green concentrated extract of 45.166 gram (9.0332%) was obtained.

3.2. Nanoherb Synthesis

In this study, sodium alginate was chosen as the polyelectrolyte compound, with calcium chloride ($CaCl_2$) as its multivalent ion. The alginate in sodium alginate was chosen for its ideal properties as a polyelectrolyte, including mucoadhesion, biocompatibility, biodegradability, non-toxicity, and affordability [25]. The Ca^{2+} in $CaCl_2$ was used due to its ability to improve the viscosity of the alginate compound and rapidly form the egg-box matrix with the guluronic acid contained in alginate [26]. When $CaCl_2$ was added into the alginate solution, a complexation reaction occurred between the carboxylate group anion in the alginate with the divalent cation Ca^{2+}, resulting in the formation of dissolved nanoherb solids [20]. The stirring was then performed to ensure that both the sodium alginate and $CaCl_2$ effectively bound the active compounds in the extract [15], [17]. Through the synthesis, a dark green aqueous colloidal solution was obtained.

3.3. FTIR Characterization

The infrared spectrum provides information regarding the functional groups and the structure of compounds contained in the nanoherb. Infrared spectral measurements were conducted on both the ethanol extract and the nanoherb solution of Tapak Liman leaves to identify differences in functional groups and compound structures before and after nanoparticle formation. The infrared spectra can be seen in Fig. 1.



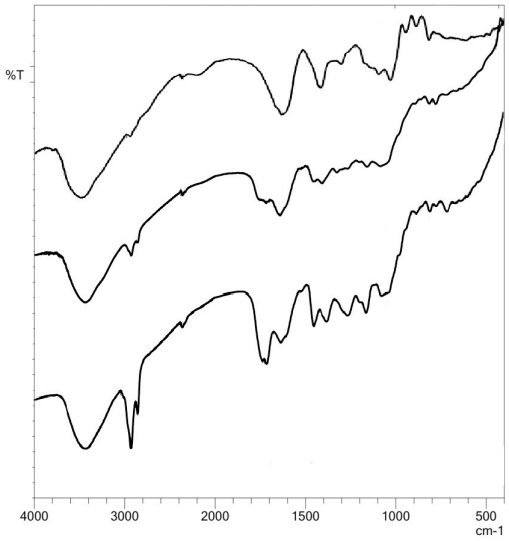


Fig. 1. Infrared spectra of (A) Extract, (B) Nanoherb, and (C) Alginate

Based on the infrared spectra, the results of the analysis presented in Table II were obtained.

TABLE II. ANALYSIS RESULTS OF INFRARED SPECTRA

Functional Groups	**Wave Number (cm^{-1})**		
	Extract	***Nanoherb***	***Alginate***
O-H	3427	3431	3452
C-H alkyl (stretching)	2927	2927	2927
C=O ketone	1715	1718	-
$-COO^-$ asymmetric carboxylic	-	1641	1632
C=C aromatic	1457	1456	-
$-COO^-$ symmetric carboxylic	-	1408	1421
C-O-C ether aliphatic	1165	1159	1127

In all three samples, wide absorption bands at 3570-3200 cm^{-1} were observed, which are attributed to O-H stretching vibration. The bands at 2935-2915 cm^{-1} are due to the C-H alkyl stretching vibration, the peaks at 1725-1705 cm^{-1} correspond to the C=O vibration of ketone groups, the bands at 1510-1450 cm^{-1} are attributed to aromatic C=C stretching vibration, the peaks at 1610-1550 cm^{-1} and 1420-1300 cm^{-1} are due to the symmetric and asymmetric vibration of $-COO^-$ group, while the peaks at 1150-1050 cm^{-1} correspond to the C-O-C stretching vibration from aliphatic ether group [27]. These results align with the research results of [28] which identified the peaks of O-H, C-H alkyl, C=O ketone, aromatic C=C, and ether C-O-C from the FTIR

measurements on encapsulation using alginate.

Overall, the results of the nanoherb FTIR spectra displayed peaks representing a combination of those found in the FTIR spectra of Tapak Liman leaf ethanol extract and alginate. This indicates the absence of a reaction between the extract and alginate, as both contribute to peaks with nearly identical wavenumbers in the nanoherb [29]. Furthermore, a decrease in amplitude of the peaks derived from the functional groups within the extract was also observed. This is likely because a significant portion of the extract is encapsulated within the egg-box matrix formed by alginate and $CaCl_2$. These findings align with those of [6] which explains that the decreased amplitude of the C=C vibration of saponin indicates its encapsulation within the long fatty acid chains of the phospholipid layers in the nanophytosome. Therefore, it can be concluded that the synthesized nanoherb encapsulated the ethanol extract of Tapak Liman leaves.

The O-H group stretching vibration in alginate exhibited shifts in the position from 3452 cm^{-1} to 3431 cm^{-1} in the ethanol extract of Tapak Liman leaves. The intensity of the peak decreased due to the interaction between the –OH groups in the sodium alginate and the –OH groups of phenolic compounds in the ethanol extract of Tapak Liman leaves [10].

The FTIR spectra of the ethanol extract of Tapak Liman leaves shows no stretching vibration in the range of 1400 cm^{-1}. This is because no bonds have been formed between the extract and alginate. During the nanoherb synthesis process, $CaCl_2$ was also used to replace the sodium ions in sodium alginate. During the synthesis, the stretching vibration of the symmetric carboxylate ion (-COONa) in alginate shifted in intensity from 1421 cm^{-1} to 1408 cm^{-1} in the ethanol extract nanoherb of Tapak Liman leaves. This happened because of the interaction of $-COO^-$ group breaking its bond with Na^+ and forming crosslinks with Ca^{2+} from $CaCl_2$ to create nanoherb. This shift of the stretching vibration to a lower wave number was because the replacement of sodium ions with calcium metal ions in sodium alginate alters the charge density, radius, and atomic weight of the cation [30].

3.4. Particle Size Characterization

From the results of measuring nanoherb particle size using Zetasizer Nano ZS, data shown in Table III were obtained.

TABLE III. PARTICLE SIZE OF ETHANOL EXTRACT NANOHERB OF TAPAK LIMAN LEAVES

Formula	*Alginate (b/v)*	*$CaCl_2$ (b/v)*	*Particle Size (nm)*
F1	0.15%	0.01%	228.3
F2	0.10%	0.01%	199.9
F3	0.05%	0.01%	184.7

In the measurement using Particle Size Analyzer (PSA), the polydispersity index data were also obtained to determine particle homogeneity. Nanoparticles with high polydispersity tend to have lower particle stability. This is because a high polydispersity index value indicates aggregation among nanoparticles, leading to heterogeneous particle sizes. Nanoparticles can be classified as homogeneous if their polydispersity index value is below 0.7 [15].

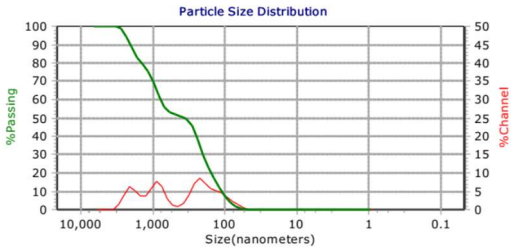


Fig. 2. Measurement results of Formula 3 particle size using PSA

The PSA data revealed that the nanoherb synthesized using Formula 3 yielded the smallest size with a polydispersity index value of 0.370, indicating that Formula 3 is the optimal formulation for nanoherb synthesis. The nanoherb was then measured for its zeta potential.

3.5. Zeta Potential Characterization

Measurement of zeta potential of the Formula 3 nanoherb resulted in a zeta potential value of 19.2 mV. This result is less than the optimal zeta potential of nanoparticles because it is smaller than +30 mV. A stable nanoparticle must have a zeta potential value less than -30 mV or greater than +30 mV [31]. This shows that the nanoherb sample has a low stability because it does not have a force to prevent agglomeration. This deviation can be attributed to its particle size. Although smaller nanoparticles can release drugs more rapidly, they also carry a higher risk of particle aggregation during storage. This aggregation is the result of sample instability [32].

IV. Conclusion

Based on the research that has been done, it can be concluded that the leaves of Tapak Liman (*Elephantopus scaber* Linn.) can be synthesized into nanoherb by the ionic gelation method using sodium alginate and calcium chloride. The synthesized nanoherb has functional groups of O-H, C-H alkyl, C=O, C=C aromatic, and C-O-C ether, with the smallest nanoparticle size of 184.7 nm, polydispersity index of 0.370, and zeta potential of 19.2 mV. The low zeta potential value, due to the small particle size, renders the nanoparticles prone to instability. The ethanol extract nanoherb of Tapak Liman leaves has the potential to be developed as a drug delivery system, contingent upon stability improvement.

References

[1] Prasetyorini, A. Rahmadini, and N. F. Utami, “Antibacterial Test of Life Leaf Extract (Gynura Procumbens (Lour.) Merr.) and Leaf Tread Liman (Elephantopus scaber L.) Against Salmonella thypi”, Ekologia: Jurnal Ilmiah Ilmu Dasar dan Lingkungan Hidup, vol. 19, no. 1, pp. 1–11, 2019.

[2] F. Rahmat, Magic Weed that Conquers Various Diseases. Yogyakarta: Penerbit Deepublish, 2020.

[3] Ifmaily and P. R. Fitriani, “The Antibacterial Mouthwash of Tapak Liman Leaves Extract (Elephanthopus scaber L) Against Streptococcus mutans”, Jurnal Medika Hutama, vol. 01, no. 02, pp. 86–91, 2020.

[4] S. W. Nasution, N. Lubis, B. C. L. Zendrato, and S. R. Silaban, “The Effectiveness of Liman Tread Leaves (Elephantopus Scaber L) Ethanol Extract Against Shigella dysenteriae Bacterial by Disc Diffusion Method”, Biospecies, vol. 14, no. 1, pp. 18–23, 2021.

[5] A. F. Esfanjani, E. Assadpour, and S. M. Jafari, “Improving the bioavailability of phenolic compounds by loading them within lipid-based nanocarriers”, Trends Food Sci Technol, vol. 76, pp. 56–66, Jun. 2018, doi: 10.1016/j.tifs.2018.04.002.

[6] Z. Nazemoroaya, M. Sarafbidabad, A. Mahdieh, D. Zeini, and B. Nyström, “Use of Saponinosomes from Ziziphus spina-christi as Anticancer Drug Carriers”, ACS Omega, vol. 7, no. 32, pp. 28421–28433, Aug. 2022, doi: 10.1021/acsomega.2c03109.

[7] M. Yousefi, M. Shadnoush, S. Sohrabvandi, N. Khorshidian, and A. M. Mortazavian, “Encapsulation Systems for Delivery of Flavonoids: A Review”, Biointerface Res Appl Chem, vol. 11, no. 6, pp. 13934–13951, Mar. 2021, doi: 10.33263/BRIAC116.1393413951.

[8] S. Sutoyo et al., "Synthesis of Nanoherbal from Ethanol Extract of Indonesian Fern Selaginella plana and Antibacterial Activity Assay", Tropical Journal of Natural Product Research, vol. 6, no. 1, pp. 44–49, Feb. 2022, doi: 10.26538/tjnpr/v6i1.9.

[9] R. A. Rahman and S. Sutoyo, "Synthesis and Characterization of Silver Nanoparticles from Ethanol Extract of Meniran (Phyllanthus niruri L.) Using Bioreduction Method", International Journal of Progressive Sciences and Technologies (IJPSAT), vol. 34, no. 2, pp. 32–40, 2022.

[10] N. Dwitarani, R. R. Amin, T. M. Sofyah, D. N. Ramadhani, and S. Sutoyo, "Synthesis and Characterization of Ethanol Extract Nanoherb of Kayu Secang (Caesalpinia sappan L.)", Jurnal Kimia Riset, vol. 6, no. 2, pp. 102–108, 2021.

[11] S. R. Mudshinge, A. B. Deore, S. Patil, and C. M. Bhalgat, "Nanoparticles: Emerging carriers for drug delivery", Saudi Pharmaceutical Journal, vol. 19, no. 3, pp. 129–141, Jul. 2011, doi: 10.1016/j.jsps.2011.04.001.

[12] S. A. A. Rizvi and A. M. Saleh, "Applications of nanoparticle systems in drug delivery technology", Saudi Pharmaceutical Journal, vol. 26, no. 1, pp. 64–70, Jan. 2018, doi: 10.1016/j.jsps.2017.10.012.

[13] Suryani, Wahyuni, D. Ariastika, and Rahmanpiu, 'Formulation of Curcumin Nanoparticles by The Ionic Gelation Technique Using Chitosan, Tripolyphosphate, and Sodium Alginate and Its In Vitro Stability Test", Pharmauho, vol. 2, no. 1, pp. 17–21, 2016.

[14] M. Yanat and K. Schroën, "Preparation methods and applications of chitosan nanoparticles; with an outlook toward reinforcement of biodegradable packaging", React Funct Polym, vol. 161, pp. 1–12, Apr. 2021, doi: 10.1016/j.reactfunctpolym.2021.104849.

[15] A. Ngafif, E. D. Ikasari, and L. W. Ariani, "Optimazation of Sodium Alginate and Calcium Chloride (CaCl2) Combination as Crosslinking Agent of Ethanol Extract Nanoparticle of Katuk (Sauropus androgynus (L.) Merr) Leaves", Berkala Ilmiah Mahasiswa Farmasi Indonesia, vol. 2, no. 1, pp. 13–23, 2020.

[16] G. M. Putri and S. Atun, "Synthesis and Characterization of Ethanol Extract Nanoparticle of Temu Kunci (Boesenbergia pandurata) in Several Variations of Alginate Composition", Jurnal Kimia Dasar, vol. 6, no. 1, pp. 19–26, 2017.

[17] L. W. Ariani and U. R. E. Purwanto, "Formulation of Extract Nanoparticles of Kembang Sepatu (Hibiscus rosa-sinensis L.) Leaves", REPOSITORY STIFAR, 2021, Accessed: Apr. 24, 2022. [Online]. Available: https://repository.stifar.ac.id/Repository/article/view/303

[18] R. Ikalinus, S. K. Widyastuti, and N. L. E. Setiasih, "Phytochemical Screening of Ethanol Extract of Kelor (Moringa oleifera) Stem Bark", Indonesia Medicus Veterinus, vol. 4, no. 1, pp. 71–79, 2015.

[19] S. Asfari, "Preparation and Characterization of Diltiazem Hydrochloride-Loaded Zinc-Pectinate Nanoparticle by Ionic Gelation Method", Thesis, Universitas Islam Negeri Syarif Hidayatullah, Jakarta, 2015.

[20] A. N. Khakim and S. Atun, "The Preparation of Kunci Pepet (Kaempferia rotunda) Extract Nanoparticles Loaded Alginate with Various Calcium Ion Concentration", Jurnal Kimia Dasar, vol. 6, no. 1, pp. 43–51, 2017.

[21] F. Azzahra, E. A. Almalik, and A. A. Sari, "Antibacterial Activity of Ethanol Extract of Avocado (Persea americana Mill.) Leaf Against Salmonella typhi and Staphylococcus aureus", AKFARINDO, vol. 4, no. 2, pp. 1–10, 2019.

[22] N. V. Wendersteyt, D. S. Wewengkang, and S. S. Abdullah, "Antimicrobial Activity Test of Extracts and Fractions of Ascidian Herdmania momus from Bangka Island Waters Likupang Against the Growth of Staphylococcus aureus, Salmonella typhimurium, and Candida albicans", Pharmacon, vol. 10, no. 1, pp. 706–712, 2021.

[23] S. Atun, "Isolation and Structure Identification Method of Organic Compounds of Natural Ingredients ", Jurnal Konservasi Cagar Budaya Borobudur, vol. 8, no. 2, pp. 53–61, 2014.

[24] N. Bahrin, N. Muhammad, N. Abdullah, B. H. A Talip, S. Jusoh, and S. W. Theng, "Effect of Processing Temperature on Antioxidant Activity of Ficus carica Leaves Extract", Journal of Science and Technology, vol. 10, no. 2, pp. 99–103, Feb. 2018, doi: 10.30880/jst.2018.10.02.016.

[25] K. T. Dewandari, G. Sofwan, and H. T., "Preparation and Characterization of Calcium Alginate Beads Containing Red Palm Oil Nanoemulation (Elaeis guineensis Jacq.) by Ionic Gelation Method", Jurnal Penelitian Pascapanen Pertanian, vol. 15, no. 2, pp. 99–105, 2018.

[26] J. Li, Y. Wu, J. He, and Y. Huang, "A new insight to the effect of calcium concentration on gelation process and physical properties of alginate films", J Mater Sci, vol. 51, no. 12, pp. 5791–5801, Jun. 2016, doi: 10.1007/s10853-016-9880-0.

[27] A. B. D. Nandiyanto, R. Oktiani, and R. Ragadhita, "How to Read and Interpret FTIR Spectroscope of Organic Material", Indonesian Journal of Science and Technology, vol. 4, no. 1, p. 97, Mar. 2019, doi: 10.17509/ijost.v4i1.15806.

[28] A. K. Pertiwi, C. Annisa, Z. Ningsih, and A. Safitri, "Microencapsulation of Ruellia tuberosa L. Extracts Using Alginate: Preparation, Biological Activities, and Release", Indonesian Journal of Chemistry. Accessed: Mar. 10, 2023. [Online]. Available: https://jurnal.ugm.ac.id/ijc/article/view/76821

[29] B. Manel, B. Youcef, B. Redhouane, and K. Affaf, "Formulation and Characterization of Beet Juice Lyophilized Microcapsules in Alginate", Research & Reviews: Journal of Chemistry, vol. 6, no. 4, pp. 49–55, 2017.

[30] H. Daemi and M. Barikani, "Synthesis and characterization of calcium alginate nanoparticles, sodium homopolymannuronate salt and its calcium nanoparticles", Scientia Iranica, vol. 19, no. 6, pp. 2023–2028, Dec. 2012, doi: 10.1016/j.scient.2012.10.005.

[31] Y. Juliantoni, W. Hajrin, and W. A. Subaidah, "Nanoparticle Formula Optimization of Juwet Seeds Extract (Syzygium cumini) using Simplex Lattice Design Method", Jurnal Biologi Tropis, vol. 20, no. 3, pp. 416–422, 2020.

[32] P. Maharani, E. D. Ikasari, U. R. E. Purwanto, and I. K. Bagiana, "Optimazation of Sodium Alginate and Calcium Chloride in Extract Nanoparticle of Purified Fucoidan from Brown Seaweed (Sargassum polycystum)", Pharmacy Medical Journal, vol. 5, no. 2, pp. 38–45, 2022.