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Original Article

Formulation and Physicochemical Characterization of Guava (*Psidium guajava* L.) Leaf Extract-Loaded Chitosan Nanoparticles

Formulasi dan Karakterisasi Fisikokimia Nanopartikel Kitosan yang Mengandung Ekstrak Daun Jambu Biji (*Psidium guajava* L.)

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Abstract

Guava (*Psidium guajava* L.) leaf extract is rich in secondary metabolites; however, its utilization as a therapeutic agent is frequently hindered by poor solubility and low bioavailability. The development of submicron-scale drug delivery systems, specifically chitosan nanoparticles, presents an approach to overcome these limitations. This study aimed to formulate and characterize chitosan nanoparticles loaded with guava leaf extract to fulfill the physicochemical stability parameters of a nanosuspension preparation. The extraction process was conducted using the maceration method with 96% ethanol. The nanoparticles were synthesized via the ionic gelation method using a 1% chitosan polymer and sodium tripolyphosphate (NaTPP) as the cross-linking agent. The characterization of the nanoparticle system included physical stability, particle size, polydispersity index (PdI), zeta potential, and morphological observation using a Transmission Electron Microscope (TEM). The freeze-dried powder was subsequently formulated into a nanosuspension, and its physical quality was evaluated. The results indicated a corrected extract yield of 26.65%. The selected nanoparticle formula (F4) exhibited an average particle size of 923.6 nm, a PdI of 0.606, and a zeta potential of +21.6 mV, demonstrating a spherical morphology but with a relatively broad particle size distribution. The evaluation of the nanosuspension over four weeks demonstrated a physically stable preparation with a pH range of 4.18–4.21, a specific gravity up to 1.0795 g/mL, and a 100% redispersion capability. In conclusion, the guava leaf extract was successfully prepared as a preliminary submicron particle formulation, demonstrating its potential to be further optimized and developed into a physicochemically stable nanosuspension.

Abstrak

Ekstrak daun jambu biji (*Psidium guajava* L.) kaya akan metabolit sekunder; namun, pemanfaatannya sebagai agen terapeutik seringkali terhambat oleh kelarutan yang buruk dan bioavailabilitas yang rendah. Pengembangan sistem penghantaran obat skala submikron, khususnya nanopartikel kitosan, menghadirkan pendekatan untuk mengatasi keterbatasan ini. Studi ini bertujuan untuk memformulasikan dan mengkarakterisasi nanopartikel kitosan yang dimuat dengan ekstrak daun jambu biji untuk memenuhi parameter stabilitas fisikokimia dari sediaan nanosuspensi. Proses ekstraksi dilakukan menggunakan metode maserasi dengan etanol 96%. Nanopartikel disintesis melalui metode gelasi ionik menggunakan polimer kitosan 1% dan natrium tripolifosfat (NaTPP) sebagai agen pengikat silang. Karakterisasi sistem nanopartikel meliputi stabilitas fisik, ukuran partikel, indeks polidispersitas (PdI), potensial zeta, dan pengamatan morfologi menggunakan Mikroskop Elektron Transmisi (TEM). Bubuk yang dikeringkan beku kemudian diformulasikan menjadi nanosuspensi, dan kualitas fisiknya dievaluasi. Hasil penelitian menunjukkan perolehan ekstrak terkoreksi sebesar 26,65%. Formula nanopartikel yang dipilih (F4) menunjukkan ukuran partikel rata-rata 923,6 nm, PdI 0,606, dan potensial zeta +21,6 mV, menunjukkan morfologi bulat tetapi dengan distribusi ukuran partikel yang relatif lebar. Evaluasi nanosuspensi selama empat minggu menunjukkan preparasi yang stabil secara fisik dengan rentang pH 4,18–4,21, berat jenis hingga 1,0795 g/mL, dan kemampuan redispersi 100%. Kesimpulannya, ekstrak daun jambu biji berhasil disiapkan sebagai formulasi partikel submikron awal, menunjukkan

potensinya untuk dioptimalkan dan dikembangkan lebih lanjut menjadi nanosuspensi yang stabil secara fisikokimia.

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INTRODUCTION

The The development of traditional therapies is currently growing, particularly those based on plants, in line with the increasing "back to nature" trend in society [1]. One tropical plant from the Myrtaceae family that shows potential for development as a therapeutic agent is guava (*Psidium guajava* L.) [2]. Various empirical studies have demonstrated that guava leaves possess a broad spectrum of pharmacological properties, including antibacterial, antioxidant, antidiabetic, and anti-inflammatory activities [3–5]. This pharmacological potential is closely related to its secondary metabolite content, such as steroids, triterpenoids, saponins, tannins, and specifically polyphenolic and flavonoid compounds[6,7].

Despite having demonstrated pharmacological efficacy, the utilization of natural extracts is frequently hindered by issues related to their fundamental biopharmaceutical properties. Specifically for guava leaf extract, its primary therapeutic constituents, such as complex tannins and specific flavonoids like quercetin exhibit low aqueous solubility, poor lipid membrane permeability, and susceptibility to presystemic degradation via environmental oxidation and gastric hydrolysis. Consequently, this specific phytochemical profile can lead to variable absorption and limited bioavailability when applied in biological systems using conventional dosage forms.

To overcome these limitations and optimize the therapeutic effects of guava leaf extract, the development of submicron-scale drug delivery systems (< 1 µm), such as nanoparticles, serves as a viable solution[8]. The use of nanocarrier technology offers various advantages, including the ability to enhance solubility, improve bioavailability, modify targeted delivery routes, and protect sensitive active substances from environmental and enzymatic degradation [9].

One type of nanoparticle carrier polymer that is frequently utilized for drug delivery is chitosan. Chitosan is a natural carbohydrate-derived polymer produced through the partial deacetylation of chitin. Its utilization is favored due to its general biocompatibility, low toxicity, and capability to form stable electrostatic interactions between the positively charged amine groups of chitosan and the negative charges of polyanionic agents [10]. This interaction facilitates the formation of nanoparticle systems via the ionic gelation method, a relatively simple technique that avoids toxic organic solvents and is capable of protecting the encapsulated bioactive compounds.

While chitosan nanoparticles have been explored for delivering isolated, single-molecule active compounds, their application for encapsulating multi-component whole plant extracts presents a specific biophysical challenge. The diverse phytochemical matrix of crude extracts can disrupt polymer cross-linking, which may lead to particle aggregation. The novelty of this study lies in optimizing the ionic gelation parameters to encapsulate the multi-component matrix of guava (*Psidium guajava* L.) leaf extract, and translating this into a physically stable liquid nanosuspension—an approach that remains limited in current literature. Therefore, this study aimed to formulate guava leaf extract into a chitosan nanoparticle system using the ionic gelation method and to perform a physicochemical

characterization. The evaluated parameters included particle size, polydispersity index, zeta potential, particle morphology, and the physical stability of the nanosuspension to ensure that this delivery system meets formulation quality requirements.

METHODS

This research was an experimental laboratory study focusing on the formulation design and physicochemical evaluation of a nanotechnological preparation. The formulation design utilized a single-factor optimization approach focused on the cross-linking agent. Specifically, four nanoparticle formulas (F1–F4) were developed by varying the volume of the 0.4% sodium tripolyphosphate (NaTPP) solution (1, 2, 3, and 4 mL) while maintaining a constant concentration and volume of the chitosan polymer. The basis for selecting these specific NaTPP volume variations was to determine the optimal stoichiometric ratio between the positively charged amine groups of chitosan and the negatively charged phosphate groups of NaTPP. This optimization is necessary to achieve the correct degree of cross-linking required to form stable submicron particles and to prevent irreversible particle aggregation. Furthermore, to ensure the reliability and reproducibility of the data, all formulation procedures and subsequent physicochemical evaluations were conducted in triplicate. All stages of formulation design and preparation characterization were carried out at the Semi-Solid Laboratory, Faculty of Pharmacy, Universitas Pancasila, Jakarta, from August to December 2024.

Materials and Instruments

The primary instrument used in the extraction stage was a rotary evaporator to concentrate the solvent. For nanoparticle characterization, high-precision instruments utilized included a Malvern Particle Size Analyzer (PSA) to measure particle size, and a Transmission Electron Microscope (TEM) to observe particle morphology. Other supporting equipment consisted of an analytical balance, sieves, an oven, a blender, glassware (Pyrex), a magnetic stirrer, a digital pH meter, a refrigerator, a pycnometer, and a freeze dryer.

The main raw material used in this study was fresh guava (*Psidium guajava* L.) leaves collected from a plantation area in Bekasi, West Java. The solvent used for the extraction process was 96% ethanol. The materials for formulating the nanoparticle system and nanosuspension included chitosan polymer, sodium tripolyphosphate (NaTPP) as a cross-linking agent, 10% dimethyl sulfoxide (DMSO), propylene glycol, Pulvis Gummi Arabici (PGA), sodium carboxymethyl cellulose (Na-CMC), 70% sorbitol, peppermint oil, benzoic acid, glacial acetic acid, and phosphate buffer at pH 6.

Plant Determination and Preparation

The guava leaves were initially determined at the UPT Laboratorium Herbal Materia Medica Batu to verify the exact plant species. The fresh leaves were cleaned and dried to reduce moisture content and prevent the growth of microbes and fungi that could degrade the quality of the active compounds [11]. The dried leaves (simplisia) were then ground and sieved using a 4/18 mesh sieve to obtain an optimal surface area, allowing for easy wetting by the solvent, in accordance with pharmacopoeial standards [12,13].

Extraction and Characterization

The The extraction of the dried powder was performed using the maceration method with 96% ethanol as the solvent. The extraction was carried out at room temperature (± 25 °C) with an initial simplicia-to-solvent ratio of 1:10. The initial maceration process was conducted for 5 days, with occasional daily stirring to facilitate the diffusion of active compounds. The process was followed by one remaceration cycle using a simplicia-to-solvent ratio of 1:5 to ensure maximum extraction. The resulting macerate was filtered, and the collected filtrate was then concentrated using a rotary evaporator at 50 °C. Subsequently, it was further concentrated over a water bath until a thick extract was formed. Based on the extraction of 1,000 g of dried powder, 266.48 g of thick extract was produced. This corresponds to a calculated extract yield of 26.65% and a DER-native (Drug Extract Ratio) of 3.75. The quality of this thick extract was subsequently evaluated through organoleptic examination, yield determination, moisture content, total ash content, residual solvent, and phytochemical screening. These parameters were measured to ensure the extract met the standard quality parameters for natural materials [14–16].

Preparation and Evaluation of Nanoparticles

The nanoparticle system was prepared using the ionic gelation method, which relies on the electrostatic interaction between the positively charged chitosan polymer and NaTPP acting as a polyanionic cross-linking agent [9,10]. The thick extract was dissolved in a mixed solvent (10% DMSO, propylene glycol, 96% ethanol, and distilled water). A 1% chitosan solution was added, followed by the dropwise addition of a 0.4% NaTPP solution under continuous magnetic stirring (200 rpm) until a stable, homogeneous turbid colloidal suspension was formed.

The evaluation of the nanoparticle preparation included determining the particle size, which ideally falls within the submicron range [17], and measuring the zeta potential to predict the electrostatic repulsive forces between particles that prevent aggregation [18]. The nanoparticle morphology was confirmed using TEM. The characterized liquid nanoparticles were subsequently dried into a powder using the freeze-drying method [19].

Formulation and Evaluation of Nanosuspension

The nanoparticle powder was formulated into a liquid nanosuspension preparation. The powder was dissolved in propylene glycol and dispersed into a suspension base consisting of a combination of suspending agents, PGA (5%) and Na-CMC (1%), which had been previously swelled with hot water. Into this mixture, 70% sorbitol, benzoic acid, peppermint oil, and phosphate buffer (pH 6) were added. The use of phosphate buffer aimed to maintain the pH range of the preparation following the addition of the benzoic acid preservative [20]. The physical quality evaluation of the nanosuspension was conducted over a four-week storage period under controlled room temperature conditions (± 25 °C). The evaluated parameters included organoleptic examination, pH measurement, specific gravity measurement for aqueous preparations [21], viscosity analysis to monitor rheological stability, as well as sedimentation volume and redispersion tests.

Data Analysis

The data obtained from the extract characterization, nanoparticle evaluation, and nanosuspension physical quality tests were analyzed using descriptive qualitative and quantitative approaches. It must be noted that all quantitative measurements—including particle size, polydispersity index (PdI), zeta potential, pH, and specific gravity—were conducted on the best formula as single observational measurements (n = 1) without technical replications. Due to this single-replication study design, the resulting data are strictly expressed as absolute single values rather than as a mean ± standard deviation (SD). All acquired data were presented in the form of tables, figures, or narratives to be

compared with established standard parameters and literature regarding the physicochemical quality of pharmaceutical preparations.

RESULT AND DISCUSSION

Plant Determination and Standardization

The initial phase of the study involved plant determination at the Herbal Materia Medica Batu Laboratory Unit, which confirmed that the specimen used was indeed guava (*Psidium guajava* L.) from the Myrtaceae family. Fresh guava leaves were dried for five days to reduce moisture content and prevent the growth of microbes and fungi, which could potentially degrade the active compounds [11]. The dried leaves were then ground and sieved using a 4/18 mesh sieve. This process ensured an optimal surface area for the powder, allowing for efficient solvent wetting during extraction, in accordance with the standards set by the Indonesian Materia Medica [12,13].

Extraction and Characterization of Guava Leaf Extract

Table 1. Phytochemical Screening Results of Guava Leaf Extract

No	Compound	Result
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Tannins	+
5	Quinones	-
6	Triterpenoids	+
7	Steroids	+
8	Coumarins	-
9	Essential Oils	-
10	Phenolics	+
11	Glycosides	+

Table 1 presents the phytochemical screening results of the guava leaf extract. The extraction was performed using the maceration method with 96% ethanol. Extraction from 1 kg of dried powder yielded 266.48 g of thick extract, representing a yield of 22.36% and a DER-native value of 4.85. Phytochemical screening identified the presence of secondary metabolites, including alkaloids, flavonoids, saponins, tannins, triterpenoids, steroids, phenolics, and glycosides. These findings align with previous studies indicating that guava leaves are rich in polyphenols and flavonoids, which serve as potential anti-inflammatory agents [5].

Quality characterization of the extract demonstrated a corrected yield of 26.65% and a corresponding DER-native of 3.75. Furthermore, the evaluation showed a moisture content of 7.65% (meeting the <10% requirement), a total ash content of 4.23%, and a residual ethanol content of 0.67%. Organoleptically, the extract appeared as a thick, dark greenish-brown mass with a characteristic odor and an astringent taste. These combined results confirm that the preparation fulfills the established standard quality parameters for thick guava leaf extract [14–16].

Formulation and Characterization of Nanoparticles

Table 2. Nanoparticle Formulation

Ingredients	F1	F2	F3	F4
Guava Leaf Extract	300 mg	300 mg	300 mg	300 mg

Dimethyl Sulfoxide (DMSO) 10%	20 mL	20 mL	20 mL	20 mL
Propylene Glycol	20 mL	20 mL	20 mL	20 mL
Ethanol 96%	20 mL	20 mL	20 mL	20 mL
Chitosan 1%	20 mL	20 mL	20 mL	20 mL
Sodium Tripolyphosphate (NaTPP) 0.4%	1 mL	2 mL	3 mL	4 mL
Distilled Water	20 mL	20 mL	20 mL	20 mL

Table 2 outlines the nanoparticle formulations. The nanoparticles were synthesized using the ionic gelation method, which relies on the electrostatic interaction between positively charged chitosan and polyanionic sodium tripolyphosphate (NaTPP) acting as a cross-linker [9].As an initial screening tool, a preliminary 5-day physical stability evaluation was conducted by observing changes in color, turbidity, and macroscopic sediment formation. Among the tested variations, Formula 4 (F4) demonstrated the most stable visual characteristics with no observable macroscopic degradation. Due to resource constraints in characterization, F1, F2, and F3 were excluded from advanced testing, and F4 was subsequently selected as the primary representative candidate for further comprehensive instrumental characterization.

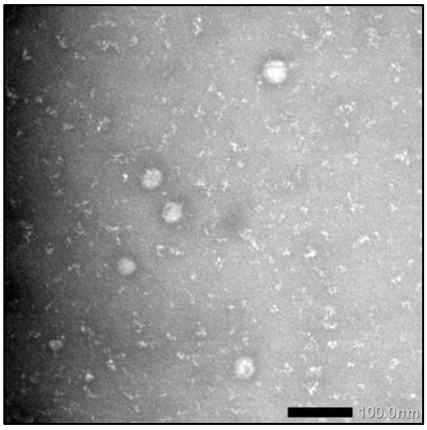


Figure 1. Morphology of Guava Leaf Extract Nanoparticles under Transmission Electron Microscope (TEM)

Figure 1 illustrates the morphology of the guava leaf extract nanoparticles. Particle size characterization using a Particle Size Analyzer revealed a mean particle size of 923.6 nm for formulation F4. While this value falls within the acceptable submicron limit for nanoparticle systems (<1000 nm), it is situated at the upper boundary of this classification [6]. Furthermore, the formulation exhibited a Polydispersity Index (PdI) of 0.606, which indicates a broad particle size distribution and a polydisperse system, rather than a strictly homogeneous dispersion [17,22]. Zeta potential analysis showed a value of +21.6 mV. This indicates a moderate positive surface charge that provides partial electrostatic repulsion; however, this moderate charge alone may not be strong enough to guarantee long-term colloidal stability against aggregation without the presence of additional steric stabilizers [18,23]. TEM observations confirmed that the particles were generally spherical. The liquid nanoparticle preparation was subsequently dried into a powder using the freeze-drying technique [19].

Physical Quality Evaluation of Nanosuspension

Table 3. Physical Stability Evaluation of the Nanosuspension Over Four Weeks

Evaluation Parameter	Week 1	Week 2	Week 3	Week 4	Observation (Over 4 Weeks)	Remarks / Reference
Organoleptic	Yellowish-brown color, minty aromatic odor	Yellowish-brown color, minty aromatic odor	Yellowish-brown color, minty aromatic odor	Yellowish-brown color, minty aromatic odor	Yellowish-brown color, minty aromatic odor	Physically stable
pH	4.21	4.20	4.20	4.18	4.18 – 4.21	Maintained by phosphate buffer
Specific gravity (g/mL)	1.0754	1.0765	1.0768	1.0795	> 1.00 g/mL	Meets aqueous vehicle criteria
Viscosity (cP)	96	91	86	82	82 – 96	Structural relaxation of polymers
Sedimentation ratio	0.05	0.05	0.10	0.10	100%	Particles settle slowly/stable
Redispersion (%)	100	100	100	100	100%	Particles redisperse perfectly upon shaking

Table 3 summarizes the physical evaluation results of the nanosuspension. The dried nanoparticle powder was formulated into a liquid nanosuspension using suspending agents consisting of a combination of Pulvis Gummi Arabici (PGA) and Sodium Carboxymethyl Cellulose (Na-CMC) to maintain dispersion stability. Physical stability testing over a 4-week period indicated that the nanosuspension exhibited a very good and stable profile overall.

Organoleptically, the preparation underwent no significant changes, maintaining the characteristic yellowish-brown color of the guava leaf extract and a minty aromatic odor from the addition of peppermint oil. The pH remained constant between 4.18 and 4.21 during storage. This acidity is primarily influenced by the benzoic acid preservative; however, the stability was successfully maintained by the phosphate buffer in the formula, preventing pH fluctuations that could degrade the active ingredients [20].

The specific gravity was consistently measured above 1.00 g/mL, reaching 1.0795 g/mL by the fourth week. These results indicate that the formulation meets the criteria for specific gravity in aqueous-based suspensions [21]. Values greater than 1.00 g/mL also indicate that the particles were successfully suspended by the added agents.

Critical parameters for suspensions include the rate of sedimentation and ease of redispersion. The sedimentation volume and redispersion tests yielded optimal results of 100%. This proves that the particles did not form

"caking" (hard sediment) but rather formed loose floccules that could be perfectly redispersed into a homogeneous suspension with light shaking.

LIMITATION

Despite the formulation and physicochemical characterization of the guava (Psidium guajava L.) leaf extract chitosan nanoparticles, this study acknowledges several important limitations. First, the botanical extract and the resulting nanoparticles were not chemically standardized based on specific active marker compounds (such as quercetin or specific tannins). Consequently, critical formulation parameters, specifically the entrapment efficiency and drug loading capacity, were not quantitatively determined. Second, while the formulated particles fell within the submicron range, the particle size distribution remained relatively broad, indicating a polydisperse system that requires further optimization to achieve strict homogeneity. Third, the physical stability evaluation of the nanosuspension was strictly conducted over a short-term period of four weeks at room temperature. The absence of accelerated stability testing under varying extreme temperature and humidity conditions means that the long-term thermodynamic stability of the colloidal system is not fully established. Fourth, the methodological scope of this study was confined to the physical and morphological parameters of the delivery system. The in vitro release profile, which is crucial for understanding the release kinetics of the entrapped bioactive compounds from the chitosan polymeric matrix, was not investigated. Furthermore, as the primary objective centered on pharmaceutical formulation, the study lacks in vivo biological assessments. Thus, the actual pharmacological efficacy, cellular uptake, and potential toxicity profiles of the formulated nanosuspension remain unverified. To address these constraints, future research should focus on chemical standardization to quantify encapsulation efficiency, refine the formulation to narrow the size distribution, and incorporate comprehensive accelerated stability studies. Additionally, in vitro drug release kinetics and in vivo pharmacological and toxicological evaluations are highly recommended to ascertain the biological efficacy, safety, and translational potential of the developed nanosuspension.

CONCLUSION

Based on the research findings and physical evaluations conducted, it can be concluded that guava (Psidium guajava L.) leaf extract was successfully prepared as a preliminary submicron particle formulation utilizing the ionic gelation method. The selected formula (F4) exhibited physical characteristics approaching the upper limits of a submicron colloidal system, with a mean particle size of 923.6 nm, a polydispersity index of 0.606, and a moderate zeta potential of +21.6 mV. While the particles possessed a generally spherical morphology, the broad size distribution indicates that the formulation requires further optimization to be considered a strictly homogeneous and mature nanoparticle system.

Furthermore, the development of these submicron particles into a liquid nanosuspension produced a formulation that maintained physical integrity over a short-term 4-week storage period at room temperature. The preparation fulfilled basic physical quality parameters, including organoleptic consistency, a stable acidic pH (4.18–4.21), suitable specific gravity (>1.00 g/mL), alongside adequate sedimentation volume and complete redispersion (100%) without caking. Overall, this study demonstrates the physical feasibility of using chitosan as a structural carrier for guava leaf extract. However, conclusions regarding its full effectiveness as a pharmaceutical delivery system remain

limited, as critical parameters—such as entrapment efficiency, active compound release profiles, in vivo bioactivity, and long-term toxicity were not evaluated and must be addressed in future studies.

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AUTHOR CONTRIBUTION STATEMENT

WP: Investigation, Data curation, Writing - Original draft preparation**; DR:** Conceptualization, Methodology, Supervision, Writing - Review & Editing**; YF:** Conceptualization, Validation, Supervision, Writing - Review & Editing.

CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest regarding the research, authorship, and/or publication of this article.

REFERENCES

1. Septianigrum A. Pengobatan kembali ke alam (back to nature) dan pemanfaatan bahan alam untuk kesehatan. *Jurnal Kesehatan Masyarakat*. 2018;5(2):44-50.
2. Jang M, Jeong SW, Cho SK, Ahn KS, Lee BH, Kim JC. Phytochemicals and biological activities of Psidium guajava. *Journal of Medicinal Plants Research*. 2013;7(10):123-131.
3. De Araújo AA, Melo MGD, Rabelo TK, Nunes PS, Santos MR V, Serafini MR. Biological activities of Psidium guajava L. *J Ethnopharmacol*. 2014;154(3):552-560.
4. Díaz-de-Cerio E, Verardo V, Gómez-Caravaca AM, Fernández-Gutiérrez A, Segura-Carretero A. Health Effects of Psidium guajava L. Leaves: An Overview of the Last Decade. *Int J Mol Sci*. 2017;18(4):897.
5. Simbolon RA, Halimatussakdiah H, Amna U. Uji Kandungan Senyawa Metabolit Sekunder pada Ekstrak Daun Jambu Biji (Psidium guajava L. var. Pomifera) dari Kota Langsa, Aceh. *Quimica: Jurnal Kimia Sains dan Terapan*. 2021;3(1):12-18.
6. Bera S, Bhattacharya M, Mitra A, Mishra S. Anti-hyperglycemic Effects of Psidium guajava LINN Crude Leaf Extracts and Fractions in Alloxan-induced Diabetic Mice. *Int J Pharm Pharm Sci*. 2013;5(3):120-125.
7. Sen A, Batra A. Study of Pharmacological Anti-inflammatory Activity of Psidium guajava Linn. Leaves and Bark by Tannin Fractions. *Research & Reviews: Journal of Pharmacognosy and Phytochemistry*. 2015;3(1):12-16.
8. Gelperina S, Kisich K, Iseman MD, Heifets L. The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis. *Am J Respir Crit Care Med*. 2005;172(12):1487-1490.
9. Abdassah M. Nanopartikel dengan gelasi ionik. *Farmaka*. 2017;15(1):45-52.
10. Winarti L, Suarmin O. Karakteristik biokompatibilitas dan interaksi kitosan dalam sistem nanopartikel. *Jurnal Ilmu Kefarmasian Indonesia*. 2011;9(1):22-29.
11. Yamin M, Hasanah U. Pengaruh metode pengeringan terhadap stabilitas senyawa aktif simplisia bahan alam. *Jurnal Teknologi Farmasi*. 2017;6(3):112-118.
12. Indonesia DKR. *Materia Medika Indonesia (Jilid I)*. Direktorat Jenderal Pengawasan Obat dan Makanan; 1977.
13. Indonesia KKR. *Farmakope Indonesia (Edisi VI)*. Kementerian Kesehatan RI; 2020.
14. Indonesia BPO dan MR. *Parameter Standar Umum Ekstrak Tumbuhan Obat (Edisi 1)*. Direktorat Jenderal Pengawasan Obat dan Makanan; 2000.
15. Indonesia KKR. *Farmakope Herbal Indonesia (Edisi II)*. Kementerian Kesehatan RI; 2017.
16. Marpaung MP, Wahyuni RC. Identifikasi dan Penetapan Kadar Flavonoid Total Ekstrak Akar Kuning (Fibraurea chloroleuca Miers). *Talenta Conference Series: Tropical Medicine (TM)*. 2018;1(3):95-98.
17. Putri TF. Sintesis dan karakterisasi nanopartikel seng oksida menggunakan ekstrak bahan alam. *Jurnal Nanoteknologi Farmasi*. 2019;2(1):11-18.
18. Indonesia BPO dan MR. *Pedoman Uji Toksisitas Nonklinik Secara in Vivo*. Peraturan Kepala BPOM RI; 2012.

19. Gaidhani KA, Harwalkar M, Bhambre D, Nirgude PS. Lyophilization/freeze drying–a review. *World J Pharm Res*. 2015;4(8):516-543.
20. Rowe RC, Sheskey PJ, Quinn ME. *Handbook of Pharmaceutical Excipients (6th Ed.)*. Pharmaceutical Press; 2009.
21. Wahyuni R, Syahrina E. Formulasi dan evaluasi stabilitas fisik sediaan suspensi dengan kombinasi suspending agent. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*. 2017;4(2):55-61.
22. Danaei M, Dehghankhold M, Ataei S, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics*. 2018;10(2):57.
23. Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems - a review (Part 1). *Tropical Journal of Pharmaceutical Research*. 2013;12(2):265-273.