



Preparation and Characterization of Betel Leaves (*Piper betle* Linn) Extract Nanoparticle with Ionic Gelation Method

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Abstract

One of the plants that can be used to improving healthy was betel leaf. Betel leaf had antibacterial and antioxidant activity. Nanoparticles are used in drug delivery which can increase mass transfer so increase the absorption and effectiveness of the drug. Therefore, its prospect to improve antibacterial and antioxidants activities of betel leaves. The research aimed to preparation and characterization of betel leaf extract using ionic gelation technique. The formulation of nanoparticles from betel leaf extract with ionic gelation method using alginate and CaCl_2 with a ratio of 2.5: 1. The characterization of the nanoparticles includes particle size analysis, zeta potential, particle morphology and determination of flavonoid content. Particle size analysis demonstrated that the betel leaf extract nanoparticles had a particle size of 243.03 ± 1.48 nm, zeta potential of -23.0 ± 0.35 mV and morphology of particle showed that a flat shape. The betel leaf extract nanoparticle positively contained flavonoid with Rf 0.7 equivalent to quercetin. The betel leaf extract can be made nanoparticles with ionic gelation method using alginate and CaCl_2 .

Keywords: Nanoparticles, Betel Leaf Extract, Ionic Gelation, PSA, SEM

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■ Introduction

The use of natural materials into herbal products is increasing because of the small side

effects and the possibility of interaction and dependence are also smaller than synthetic drugs. One of the plants used is betel leaf (*Piper betle* Linn). Betel leaves have antibacterial activity [1-4]

and antioxidants [5, 6]. Ethanol extract 96% of betel leaf also has antibacterial activity against *Staphylococcus aureus* in 2.5% MIC (Minimum Inhibitory Level) with disk diffusion method [4]. Betel leaves extract contains eugenol, dimethylbenzoic acid, decahydro-4a-methyl-1-methylenynaphthalene 1,2,3,4,4a,5,6,8a-octahydro-7-methyl naphthalene and 1,2,3,4,4a,5,6,8a-octahydro-4a-methyl naphthalene [7]. Active compounds that have antibacterial activity are flavonoids.

Flavonoids instable at high heating and high light intensity which changes flavonoids structure and reduce its activity [5, 6]. Flavonoids in plants have several health benefits, including antioxidants and antihypercholesterolemia as well as modulators of cell signaling and gene expression. Bioavailability of flavonoids was low and this would be a determinant factor of flavonoids bioactivity *in vivo* [8]. Numerous attempts have been made to increase bioavailability, such as improving the intestinal absorption, novel delivery system, reducing particle size and solid dispersion. Nanotechnology is the conversion of materials into small particle sizes using physical, chemical and biological methods [9]. Nanoparticles can increase mass transfer so that it can increase the absorption and effectiveness of the drug [10]. In the research showed that Ag-proanthocyanidin nanoparticles can increase the effectiveness of antibacterial and antifungal and have good stability [11]. Nanoparticles technology can be used to maintain and protect the active compounds.

The advantages of nanoparticles technology include increasing stability and long-term storage. Nanoparticles can increase the solubility of active compounds, reduce therapeutic doses and increase absorption of herbal medicines [12]. The synthesis of nanoparticles based on polymers can be used polymer dispersion, polymerization of monomers and ionic gelation methods. Ionic gelation technique is the easiest method compared to other methods, has a good biocompatibility, easy method application, does not require organic solvents in large quantities so it is relatively cheaper [13]. In ionic gelation methods, particles are formed in a single step by a simple mechanism, usually involving two different polymers and one complexation agent, by adding one polymer solution to the other one with stirring.

Most commonly described complexation agents used with chitosan and alginate are calcium chloride and tripolyphosphate (TPP) [14].

Alginate is natural polymer that is mucoadhesive, biodegradable and biocompatible, enabling numerous pharmaceutical and biomedical applications such as drug delivery and cell encapsulation. An important property toward the application of alginate for nanoencapsulation of drugs is the fact that the polymer sol forms a reversible gel when ionically crosslinked with multivalent cations such as Ca^{2+} , enabling drug retention within the gel matrix. The interaction between alginate in dilute solution with Ca^{2+} occurs at a certain ion concentration. A pre-gel state results with stirring, avoiding the gel point, forming a continuous system [15].

In some researches have shown that plant extract can be made nanoparticles. Fingerroot extract could be made into nanoparticles using alginic acid: CaCl_2 (2.5: 1) with particle sizes 399-877 nm [16]. Curcuma extract can be made nanoparticles by ultrasonic with particles size 470-3000 nm [17]. Utilization of betel leaf in herbal medicine is still in crude extracts so betel leaf extract nanoparticles are made with ionic gelation method.

Based on the background above, a study was conducted to fabrication and evaluation of betel leaf ethanol extract nanoparticles using alginate and CaCl_2 as polymer with ionic gelation method.

■ Experimental Section

Materials

Betel leaves obtained from Giritirto, Wonogiri in April 2019, ethanol 70% (CV Liquid Pharmalab, BN: 19011906), ethanol p.a (Mallinckrodt, CAS NO: 64-17-5), Na alginate, CaCl_2 , aquadest, methanol p.a (Merck, Emsure, CAS-NO: 67-56-1), chloroform p.a. (Merck, Emsure, CAS-NO: 67-66-3)

Instrumentation

Rotary Evaporator (IKA RV 10 Basic), Particle Size Analyzer (HORIBA SZ-100), Zeta Sizer (HORIBA SZ-100), Scanning Electron Microscopy (JED-2300, 9510 LA), and Thin Layer Chromatography plate (Silica Gel 60 F₂₅₄).

Methods

Extraction of Betel Leaves

The amount of 200 g of betel leaf dry powder was macerated with 1 L 70% ethanol (1x24 hours). The filtrate obtained was accommodated and the pulp was macerated again with 0.5 L 70% ethanol (1x24hours). Then the filtrate obtained was accommodated again and the pulp is macerated again with 0.5 L 70% ethanol (1 x 24 hours). The filtrate was collected and concentrated with a rotary evaporator until the thick extracts were obtained.

Preparation of Leaf Betel Extract Nanoparticles

The betel leaf extract (1 g) was dissolved in 35 mL ethanol p.a and added 15 mL of distilled water. In the mixture, 0.1% alginate solution (alginic acid powder dissolved in 0.1 M NaOH) was added as much as 100 mL and 0.02% CaCl_2 solution as much as 350 mL using a magnetic stirrer for 2 hours. The formed colloids werw centrifuged and taken soluble solids, which are then washed with distilled water and dried in the freezer for 2 days and then stored in a refrigerator until it becomes dry powder [16; 18]

Characterization of Nanoparticles

Colloid nanoparticles were characterized using Particle Size Analysis (PSA) to determine particle size. Zeta potential was measured by Zeta Sizer. The particle morphology was characterized by using Scanning Electronic Microscopy.

Thin Layer Chromatography (TLC)

Qualitative analysis of flavonoid compounds was determine by using quercetin as a reference. Thin Layer Chromatography method was perfomed to analyze the flavonoids compound with silica gel as stationary phase and UV detectors at 254 and 366 nm and mobile phase was used methanol:chloroform (9:1). A total of 10 mg of quercetin was diluted with 10 mL methanol p.a. samples of betel leaf nanoparticle extract were weighed and dissolved in methanol p.a.

The reference and the sample solution were bottled in a stationary phase then incorporated into a vessel saturated with a mobile phase and covered with glass. Stain spots on the TLC plate were observed on UV detectors at 254 nm and 366 nm.

The Rf value is calculated from the stain spot obtained.

■ Results and Discussion

Extraction of betel leaves using 70% ethanol to take flavonoids that was the antibacterial activity compounds in betel leaves. Flavonoids was the secondary metabolite of betel leaves and include under the broad category of polyphenols [8]. The method used is maceration because it is a simple, inexpensive and suitable for compounds that instable to high heating. The organoleptics of extract obtained was thick extract, blackish green color and has characteristic odor of betel leaf. The extract yield obtained was 13.66%. This result is smaller than using the infundation method with water solvents. The exctract yield obtained from infundation method was 16.03% [3].

Bioavailability of flavonoids generally was low bioavailability and can vary drastically among different flavonoid classes as well as individual compounds in a particular class [8]. To Increase bioavailability of flavonoids in betel leaves extract was made nanoparticles. Nanoparticles had smaller particle size so increased membran permeability and intestinal absoprtion. The manufacture of nanoparticles using ionic gelation method because it is the easiest method among the other methods. Ionic gelation is made from the polymer alginate and CaCl_2 with a ratio of 2.5: 1, because this ratio had produced the smallest nanoparticles of fingerroot extract [16]. The use of alginate as a polymer in the manufacture of nanoparticles because alginate is a polymer that is often used in drug-carrying systems and has non-toxic, biocompatible and biodegradable properties [19, 20]. The use of CaCl_2 together with alginate will form crosslinking so that it can increase molecular weight. Crosslinking polymers formed have properties that are insoluble in water but have a pH-dependent permeability to water vapor [21].

The bond between Ca^{2+} ions and alginate will produce deposits which are complexations of Na^+ ions with divalent ions from CaCl_2 [19]. In the process of dissolving alginate, decomplexation occurs due to the release of Na^+ ions and ionic alginate structures, and in the presence of CaCl_2 there will be complexations that form deposits. To remove the remaining Cl^- , the precipitate is washed with distilled water. Sediment formed Ca^{2+}

ions are used as crosslinking agents because they have faster alginate gel formation rates than other divalent ions [19].

The betel leaves extract nanoparticles had a particle size of $243,03 \pm 1,48$ nm which showed the particles are in the size of a nanometer (<1000 nm). The size and distribution of particle size can be used to estimate distribution in vivo, biology, toxicity and the ability to reach the target of the nanoparticle system [22]. By knowing the size and distribution of particle size can know firsthand the unique nature of nanoparticles [23]. At the research showed that fingerroot extract can be made nanoparticles by ionic gelation method with particle size of 100-776 nm [18]. Small particles

size have a large surface area and most of drug will be around the surface of the particle, so drug release is faster [23]. The concept of ionic gelation can use of two kinds of biopolymers in one formulation system. The two biopolymers used must have opposite charges, so they can form a flexible matrix for absorbing drugs with wider properties [24].

The betel leaf extract nanoparticle had zeta potential of $-23,0 \pm 0,35$ mV. Nanoparticles with zeta potential values less than -30 mV and more than 30 mV have better stability [25]. A high zeta potential value of either positive or negative indicates a high electrostatic force so as to prevent phase separation [26].

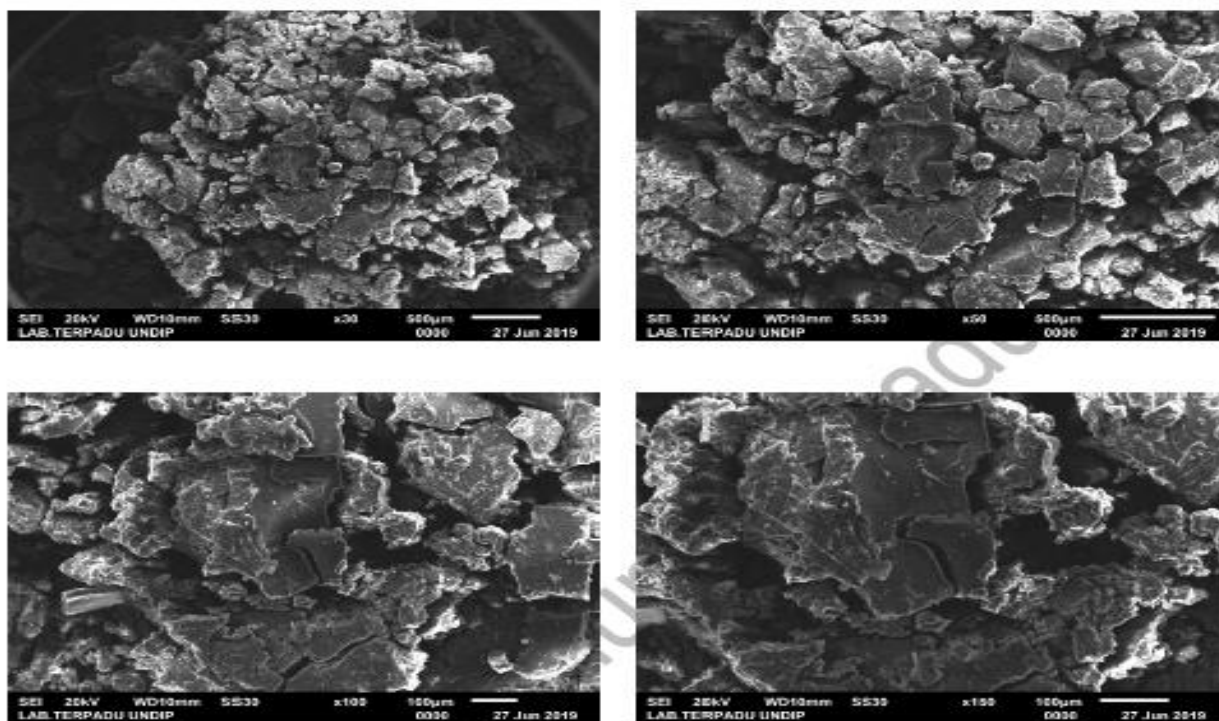


Figure 1. SEM testing result of nanoparticles of betel leaves extract

The SEM test is efficient to determine images of sample surfaces. The way this microscope works is by transmitting electrons to the surface of the sample. The introduction of probes in electron beam currents that hit the surface of particles can provide information about the surface of the particles which is then carried by a probe in the tunnel between the surface of the particle and the

probe tip [27]. The results of SEM testing of betel leaf extract nanoparticles can be seen that the particles are flat as shown in figure 1. SEM test results showed a flat-shaped particles. Particle morphology is important because the less spherical of particles shape are will facilitate contact between particles to lead to aggregation [24].



Figure 2. TLC testing result of nanoparticles of betel leaves extract

The next characterization of the betel leaf extract nanoparticles is Thin Layer Chromatography (TLC) testing to determine the content of the active compound by using a quercetin as standard. The results of TLC testing of nanoparticles of betel leaves extract can be seen in figure 2. The results showed that between the betel leaf extract nanoparticles has the same Rf value as the comparative standard which is 0.7. This shows that betel leaf extract nanoparticles positively contain flavonoids. The Rf price that approaches each other shows that in betel leaf extract nanoparticles there is a quercetin content which is a marker of flavonoids [28].

■ Conclusion

Betel leaf extract can be made nanoparticles by ionic gelation method with Na alginate and CaCl_2 as polymer. Betel leaf extract nanoparticles had particle size of $243,03 \pm 1,48$ nm, zeta potential of $-23,0 \pm 0,35$ mV and had a flat-shaped particle morphology. Betel leaf extract nanoparticles had flavonoid compounds.

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■ References

- [1] Ahmad dan Suryana, 2009, Pengujian Aktivitas Ekstrak Daun Sirih (*Piper betle* Linn) Secara In Vitro, IPB, Bogor, *Buletin Littro*, Vol 20, No 1, 92-98.
- [2] Hermawan, A., Eliyani, H., Tyasningsih, W., 2007, Pengaruh Ekstrak Daun Sirih (*Piper betle* Linn) Terhadap Pertumbuhan *Staphylococcus aureus* dan *Eschericia coli* Dengan Metode Difusi Disk, *Artikel Ilmiah*, Fakultas Kedokteran, Universitas Airlangga, Surabaya
- [3] Mufrod, Suwaldi, Wahyuono, S., 2016, Patch Ekstrak Daun Sirih (*Piper betle* Linn): Pengaruh Penambahan *Release Enhancer Substances* Terhadap Sifat Fisikokimia dan Aktivitas Antibakteri, *Majalah Farmaseutik*, Vol 12, No 2
- [4] Putri, Z.F., 2010. Uji Aktivitas Antibakteri Ekstrak Etanol Daun Sirih (*Piper betle* L.) Terhadap *Propionibacterium acne* Dan *Staphylococcus aureus* Multiresisten, *Skripsi (S1)*, Universitas Muhammadiyah Surakarta
- [5] Andarwulan, N., Wijaya, C.H., Cahyono, D.T., 1996. *Aktivitas antioksidan dari daun sirih (Piper betle L.)*
- [6] Parwata, I., Rita, W.S., Yoga, R., 2009. Isolasi dan uji antiradikal bebas minyak atsiri pada daun sirih (*Piper betle* Linn) secara spektroskopi ultra violet-tampak. *J. Kim.* 3, 7–13
- [7] Pratiwi, N.P.R.K., and Muderawan, I.W., 2016, *Prosiding Seminar Nasional MIPA*, FMIPA, Undhiksa
- [8] Thilakarathna, S.H., and Rupasinghe, H.P.V., 2013., Review: Flavonoid Bioavailability and Attempts for Bioavailability Enhancement, *Nutrients*, 5, 3367-3387.
- [9] Debnath, P., Mondal, A., Hajra, A., Das, C., Mondal, N.K., 2018, Cytogenetic Effects of Silver and Gold Nanoparticles on *Allium cepa* roots, *Journal of Genetic Engineering and Biotechnology*
- [10] Amani, P., Amani, M., Ahmadi, G., Mahian, O., Wongwises, S., 2018. A critical review on the use of nanoparticles in liquid–liquid extraction. *Chem. Eng. Sci.* 183, 148–176. <https://doi.org/10.1016/j.ces.2018.03.001>
- [11] Biao, L., Tan, S., Zhang, X., Gao, J., Liu, Z., Fu, Y., 2018. Synthesis and characterization of proanthocyanidins-functionalized Ag nanoparticles. *Colloids Surf. B Biointerfaces* 169, 438–443
- [12] Goyal, et al, 2011, Potential of Novel Drug Delivery Systems for Herbal Drugs, *Indian Journal of Pharmaceutical Education and Research*, 45 (3), 225-235

- [13] Saraei, et al, 2013, Design and Evaluate Alginate Nanoparticles as a Protein Delivery System, *Archives of Razi Institute*, 68 (2), 139-146
- [14] Caetano, L.A., Almeida, A.J and Goncalves, L.M.D., 2016, Effect of Experimental Parameters on Alginate/Chitosan Microparticles for BCG Encapsulation, *Marine drugs*, 14,90
- [15] Sarmiento, B., Ribeiro, A.J., Veiga, F., Ferreira, D.C., Neufeld, R.J., 2007, Insulin-Loaded Nanoparticles are Prepared by Alginate Ionotropic Pre-Gelation Followed by Chitosan Polyelectrolyte Complexation, *Journal of Nanoscience and Nanotechnology*, Vol 7, 1-9
- [16] Putri, G.M., dan Atun, S., 2017, Pembuatan dan Karakteristik Nanopartikel Ekstrak Etanol Temu Kunci (*Boesenbergia pandurata*) Pada Berbagai Variasi Komposisi Alginat, *Jurnal Kimia Dasar*, Vol 5, No 1
- [17] Sidqi, T., 2011, Pembuatan dan Karakterisasi Nanopartikel Ekstrak Temulawak Dengan Metode Ultrasonikasi, *Skripsi*, Departemen Biokimia Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Pertanian Bogor
- [18] Khakim, A.N., dan Atun, S., 2017, Pembuatan Nanopartikel Ekstrak Kunci Pepet (*Kaempferia rotunda*) Dengan Alginat Pada Berbagai Variasi Konsentrasi Ion Kalsium, *Jurnal Kimia Dasar*, Vol 6, No 1
- [19] Umawiranda, P.S., dan Cahyaningrum, S.E., 2014, Enkapsulasi Pirazinamid Menggunakan Alginat dan Kitosan. *Journal of Chemistry*, Volume 3 No 3
- [20] George, M., and Abraham, E., 2006, Polyionic Hydrocolloids for the Intestinal Delivery of Protein Drugs: Alginat and Chitosan – a Review, *Journal of Controlled Release*, 114(1), 1-14
- [21] Rowe, R.C., Sheskey, P.J., dan Quinn, M.E., 2009. *Handbook Of Pharmaceutical Exipients*, 6th ed. Pharmaceutical Press and American Pharmacists Association, London,UK.
- [22] Mohanraj, V.J., and Y.Chen., 2006, Nanoparticles: A Review, *Tropical Journal of Pharmaceutical Research*, Vol. 5 No 1
- [23] Abdassah, M., 2017, Nanopartikel Dengan Gelasi Ionik, *Farmaka*, Volume 15 No 1, 45-52
- [24] Martien, R., Adhyatmika, Irianto, I.D.K., Farida, V., Sari, D.P., (2012), Perkembangan Teknologi Nanopartikel Sebagai Sistem Penghantaran Obat, *Majalah Farmaseutika*, Volume 8, No 1.
- [25] Murdock, et al, 2008, Characterization of Nanomaterial Dispersion in Solution Prior to In Vitro Exposure Using Dynamix Light Scattering Technique, *Toxicological Sciences*, 101 (2), 239-253
- [26] Avachat, A.M. dan Patel, V.G., 2014. Self nanoemulsifying drug delivery system of stabilized ellagic acid–phospholipid complex with improved dissolution and permeability. *Saudi Pharmaceutical Journal*
- [27] Poole CRJr and Owens FJ., 2003, *Introduction to Nanotechnology*, New Jersey: John Wiley & Sons Inc.
- [28] Pertiwi, R.D., Yari, C.E., Putra, N. F. 2017. Uji Aktivitas Antioksidan Ekstrak Etanol Limbah Kulit Buah Apel (*Malus domestica* Borkh) Terhadap Radikal Bebas DPPH (2,2-Diphenyl -1-Picrylhydrazil). *Jurnal Ilmiah Manuntung*, 2, Akademi Farmasi Samarinda. Samarinda