ICCSCP Atlantis Press Journal

by Herlina Herlina

Submission date: 07-Jun-2023 12:04PM (UTC+0700)
Submission ID: 2110805550
File name: ICCSCP_DINA_PERMATA_WIJAYA.pdf (322.75K)
Word count: 3278
Character count: 18454

Advances in Health Sciences Research, volume 40
2nd International Conference on Contemporary Science and Clinical Pharmacy 2021 (ICCSCP 2021)

Characterization and Antioxidant Activity of Nanoparticles Loaded Jackfruit Leaf Extract (*Artocarpus heterophyllus* Lamk.)

Dina Permata Wijaya[1*] Mardiyanto[1] Herlina[1] Vabiola Besti Delmoda[1]

[1]*Department of Pharmacy, Faculty of Mathematics and Sciences, Sriwijaya University, Indonesia*
[*]*Corresponding author. Email: dinapermatawijaya@unsri.ac.id*

ABSTRACT

Jackfruit leaves extract (*Artocarpus heterophyllus* Lamk.) which contained flavonoid as an antioxidant was unstable and sensitive to environmental conditions. Preparation of nanoparticles loaded jackfruit leaf extract aimed to increase the antioxidant activity through optimal delivery system and maintain the stability of flavonoid from jackfruit leaf extract. Nanoparticle preparation used an ionic gelation method with chitosan, sodium alginate, and variation of calcium chloride. Characterization of nanoparticles were particle size, polydispersity index, efficiency of encapsulation, zeta potential, and XRD analysis. Based on the result, the highest percent of encapsulation efficiency in the nanoparticles with the amount of calcium chloride 40 μl was 90,76%. The characterization of nanoparticles, particle size was 244,2 nm, polydispersity index was 0,22, and zeta potential was +26,4 mV. The XRD pattern of nanoparticles loaded jackfruit leaf extract showed jackfruit leaf extract encapsulated into nanoparticles. Antioxidant activity of jackfruit leaf extract increased after encapsulation as indicated by the IC_{50} for nanoparticles of jackfruit leaf extract was 10,13μg/ml which indicates high antioxidant.

Keywords: *antioxidant, chitosan, DPPH, jackfruit leaf extract, nanoparticles.*

1. INTRODUCTION

Nanoparticles are particle technology that aims to change or modify particle size to nanometer size that it can facilitate absorption and increase its effectiveness [1]. Nanoparticles term have a particle size range 200-500 nm that have been widely used in the pharmaceutical field and have advantages in drug delivery, namely they can overcome the solubility of poorly soluble active substances, improve poor bioavailability, modify drug delivery systems so that drugs can go directly to specific targets, and increase the stability of the active substance from the environment [2]. Nanoparticle technology can be used to help penetrate the drug into the skin because the particle size is in nanometer makes to easier for the drug to penetrate the skin layers and can help increasing the protective effect of the drug so that it is not easily degraded [3].

Nanoparticles can be applied to deliver the active substance by encapsulated. The active substance is encapsulated into the nanoparticle by using a polymer. Several polymers that can be used for nanoparticle systems are chitosan, gelatin, albumin, and sodium alginate [4].

Nanoparticles formed from particle molecules have several advantages, including non-toxic and stable, as well as biodegradable and biocompatible. Chitosan can also interact by opening tight junctions between cells so that it can increase drug transport into cells. In addition, chitosan also has the advantage of being able to control the release of the encapsulated active substance [5]. Sodium alginate can be used as a polymer for preparation of nanoparticles. The preparation of nanoparticles can use a combination of chitosan and sodium alginate to form complex poly ion bond that can encapsulate the extract [6]. Sodium alginate also nontoxic, biodegradable, and biocompatible properties which can also be used as food additives, gelling agents, emulsifiers, and stabilizers [7]. In nanoparticle system, a cross linker is needed which will interact with sodium alginate to form a polyelectrolyte complex. Calcium chloride will interact with carboxylate group on sodium alginate. The complex formed will strengthen the interaction between the polycation and polyanion of chitosan and sodium alginate so that it is able to coat the active substance more effectively [8].

One of the plants from Indonesia that used for food is Jacfruit (*Artocarpus heterophyllus* Lamk.). In addition to the fruit, jackfruit leaves can be used as medicinal plants because they contain flavonoids, phenols, steroids, and

tannins. Flavonoids have activity as an antioxidant, antiinflammatory, antifungal, antiviral, anticancer, and antibacterial. Based on research the content of quercetin contained in jackfruit leaf extract is 18,48%. Quercetin is a compound that belongs to the flavonoid group which has antioxidant activity of jackfruit leaf extract using DPPH method resulted in a stronger antioxidant activity than jackfruit seed extract, which was 73,5 g/ml [9,10]. However, the use of flavonoids for treatment has disadvantages, namely low solubility, unstable to environmental conditions, metabolized by intestinal microflora, and low bioavailability [11].

Based on the above, this study aims to prepare, characterize, and test the antioxidant activity of nanoparticles loaded jackfruit leaf extract using chitosan and sodium alginate polymers with variation of calcium chloride as a crosslinker.

2. METHODS

2.1. Tools

The tools that used in this study were analytical balances (Ohaus®), UV-Vis spectrophotometer (Biobase® BK-UV1900PC), glassware (Pyrex®), *bath sonicator* (Elmasonic® S 180 H), sentrifugasi (InScienPro® Xlab 04), micro pipette (DragonLab®), Vivaspin® 300 kDa, and PSA (Horiba Scientific®).

2.2 Materials

The materials used in this study were jackfruit leaf simplicia (*Artocarpus heterophyllus* Lamk.) determined by the Purwodadi Botanical Gardens LIPI, ethanol 96% (PT. Dira Sonita®), Aqua Pro Injection (PT. Dira Sonita®), citric acid (PT. Bratachem®), calcium chloride (PT. Merck®), chitosan (CV. ChiMultiguna®), sodium alginate (Nusae), quercetin (PT. Merck®), methanol p.a (PT. Merck®), sodium acetate (PT. Merck®), silica gel TLC plate GF_{254}, Whatman filter paper and aluminum chloride ($AlCl_3$).

2.3 Extraction of Jackfruit Leaf

Jackfruit leaf extraction was carried out using the maceration method. A total of 400 g of simplicia of jackfruit leaves was macerated using 2 L of 96% ethanol and allowed it to stay for 3 days with some stirring. The maceration results were filtered using filter paper to produce a filtrate. The residue was macerated again so that a clear filtrate is obtained. The resulting filtrate was evaporated using an evaporator at a temperature of 60°C at a speed of 35 rpm to remove solvent and produce a viscous extract. The resulting viscous extract was identified and measured the total amount of flavonoids produced using quercetin as a comparison compound.

2.4 Formulation of Nanoparticles Loaded Jackfruit Leaf Extract

The formulation of nanoparticles loaded jackfruit leaf extract in this study varied the amount of $CaCl_2$ (Table 1). Nanoparticles loaded jackfruit leaf extract were prepared using the ionic gelation method. A total of 111 mg of jackfruit leaf extract was dissolved in 3 ml of ethanol then the extract solution was dissolved into 7 ml of dissolved chitosan using 2% citric acid using a magnetic stirrer at 750 rpm for 10 minutes. Then, 10 ml of sodium alginate solution was dropped into the chitosan solution and the extract was stirred using a magnetic stirrer for 30 minutes at a speed of 750 rpm. After being homogeneous, the calcium chloride solution was added by drop by drop while stirring with a magnetic stirrer for 30 minutes at a speed of 750 rpm. Then the resulting nanoparticles loaded jackfruit leaf extract were sonicated with a frequency of 50 kHz for 30 minutes.

Table 1. Formulation of Nanoparticles Loaded Jackfruit Leaf Extract (*Artocarpus heterophyllus*)

Formula	Extract (mg)	Chitosan (mg)	Sodium Alginate (mg)	$CaCl_2$ (μl)
F1	111	12	3,2	20
F2	111	12	3,2	40
F3	111	12	3,2	100

2.5 Determination of Percent Encapsulation Efficiency (%EE)

Analysis of the percent efficiency of encapsulation used nanoparticles loaded jackfruit leaf extract that being centrifuged at 12,000 rpm for 15 minutes. The resulting supernatant was measured for flavonoid content using a UV-Vis spectrophotometer with a wavelength of 436 nm which used for quercetin as a marker compound.

2.6 Characterization of Nanoparticles Loaded Jackfruit Leaf

The particles size, polydispersity index (PDI), and zeta potential were measured using a Particle Size Analyzer (PSA) with dynamic light scattering (DLS) method. A total of 50 μl of nanoparticles loaded jackfruit leaf extract diluted 100 times to 5 mL using distilled water, then 50 μl solution were added to the PSA cuvette and then measured.

2.7 Determination of Antioxidant Activity

The antioxidant activity of nanoparticles loaded jackfruit leaf extract was determined based on the method of Loizzo, 2009 [12]. A total of 1.0 mL of 0.3 mM DPPH

solution in methanol and the concentration ranges of jackfruit leaf extract used were 600, 650, 700, 750, 800 μg/ml.
A total of 2 ml of 0.3 mM DPPH solution added 2 ml of sample and allowed to stand for 30 minutes in a dark room. The absorbance was measured using a UV-Vis spectrophotometer with a maximum DPPH length of 517 nm. Antioxidant activity in the test solution is determined by the amount of DPPH that is inhibited.

$$\% \text{ inhibition} = \frac{control\ absorbance - Sample\ absorbance}{absorbance\ control} \text{ x } 100\%$$

3. RESULTS AND DISCUSSION

The process of extracting jackfruit leaves using the maceration method produced a thick extract of 56.65 g with a percent yield of 14.16%. Maceration method is used in this research because maceration belongs to the cold extraction method using a certain solvent. The selection of this method is based on the condition of flavonoid compounds which are not resistant to heat and easily oxidized at high temperatures (>90°C).
Determination of the total flavonoid content of jackfruit leaf extract using quercetin as a comparison. The total flavonoid produced by jackfruit leaf extract was 23.18 mg/g. It was determined that the total flavonoid content in the nanoparticles loaded jackfruit leaf extract was 2.33 mg/111 mg (Table 4).

Table 4. Flavonoid Content Total

Sample	Total Flavonoid (mg/111mg)
Extract	2,57
F1	2,25
F2	2,33
F3	2,27

Nanoparticles loaded jackfruit leaf extract were formulated using a combination of chitosan polymer and sodium alginate. The method used in the manufacture of nanoparticles loaded jackfruit leaf extract is the ionic gelation method which has the basic principle of electrostatic interaction between different charged groups of polymers and crosslinkers. The ionic gelation method in the dissolving process does not use organic solvents so it is safer to use. The polymer used is chitosan and sodium alginate which will cause an ionic interaction between the polycation in chitosan and polyanion in sodium alginate. In this method there is also an electrostatic interaction that occurs between the negative charge of the polyanion on the sodium alginate and the positive charge of the amine on the chitosan[13].

The cross linker used in the manufacture of nanoparticles is calcium chloride ($CaCl_2$) which is useful for forming

The IC_{50} value was calculated using the regression method [12]. Antioxidant test control for this study used quercetin as a comparison. The antioxidant activity (Table 3)

Table 3. IC_{50} Value of Antioxidant Activity

Sample	IC_{50}	Antioxidant Activity
Quercetin	8,68 μg/ml±0,167	Very Strong
Extract	27,45 μg/ml±0,234	Very Strong
Nanoparticle	11,56 μg/ml±0,134	Very Strong

polyelectrolyte complexes with sodium alginate. Calcium chloride will interact with the carboxyl group of sodium alginate. Sodium ions will be replaced by calcium ions and form a three-dimensional structure in sodium alginate[4]. The complex formed will strengthen the interaction between the polycation and polyanion of chitosan and sodium alginate so that the coating ability is maximized.
The preparation of nanoparticles loaded jackfruit leaf extract has an organoleptic color with a brownish yellow and opaque/cloudy color. The total volume nanoparticles loaded the jackfruit leaf extract was 20 ml. The preparation of jackfruit leaf extract nanoparticles was being characterized.
Percent encapsulation efficiency aims to determine the amount of jackfruit leaf extract that is encapsulated into nanoparticle vesicles. The results of the percent efficiency measurement show that formula 2 has the highest %EE while formula 3 produces the smallest %EE. The results of the percent encapsulation efficiency of each formula can be seen in Table 2.

Table 2. Percent of Encapsulation Efficiency Nanoparticles Loaded Jackfruit Leaf Extract

Formula	%EE±CV
F1	89,48%±0,135
F2	90,74% ±0,034
F3	88,12%±0,068

The concentration of calcium chloride used affects the percent encapsulation because it will strengthen the electrostatic bond that occurs in the two polymers. The higher %X value indicates that more extracts are encapsulated in the nanoparticle system. The nanoparticles preparation with the highest percent encapsulation efficiency was characterized (formula 2). Characterization of nanoparticles loaded jackfruit leaf extract can be seen in table 5.

Table 5. Characterization of Nanoparticles Loaded Jackfruit Leaf Extract

Characterization	Result
Particles Size	244,2 nm
PDI	0,22
Zeta Potential (mV)	+26,4 mV

Particles diameter and distribution are important parameters to be measured in nanoparticle vesicle systems. This parameter will affect the ability of nanoparticle vesicles to penetrate the skin[14]. The diameter of the particles produced by the nanoparticles loaded jackfruit leaf extract was 244,2 nm. Particles with a small diameter will have a large particle surface so that their solubility is good and their bioavailability is increased[15]. The polydispersity index value of nanoparticles loaded jackfruit leaf extract was 0,22 which indicated that 78% of the total number of nanoparticles loaded jackfruit leaf extract had homogeneous sizes. The polydispersity index value of nanoparticles loaded jackfruit leaf extract has a value of <0.5 which indicates that the diameter size distribution is uniform. The particle diameter and polydispersity index value in the nanoparticles loaded jackfruit leaf extract were in accordance with the objectives, namely in the range of 200-500 nm with a polydispersity index <0.5.

The zeta potential measurement shows the surface charge of the particles which indicates the stability of the particles during storage. The stable zeta potential has a value of ± 25 mV[16]. The potential zeta value produced by nanoparticles loaded jackfruit leaf extract is +26,4 mV. The potential zeta value of the nanoparticles loaded jackfruit leaf extract has a positive surface charge. This caused by chitosan that has a positively charged amino group resulting in a positive zeta potential.

The XRD pattern of the jackfruit leaf extract showed a change to an amorphous form. Amorphous forms do not have peaks that are separated at a certain distance and the resulting pattern is wide, while the crystalline form has peaks that are separated at a certain distance and the resulting pattern is not wide. This research is in accordance with what is expected, namely the particles must be amorphous because in that form the particles are more soluble and quickly absorbed[14]. The XRD spectrum can be seen in Figure 1.

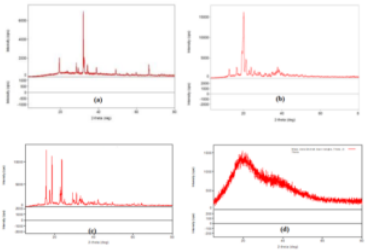

Figure 1. The Pattern XRD of (a) chitosan (b) sodium alginate (c) jackfruit leaf extract (d) nanoparticles loaded jackfruit leaf extract

The antioxidant activity of nanoparticles loaded jackfruit leaf extract using the DPPH method. The purpose of this method is to determine the concentration of the test solution that can inhibit 50% of free radicals (IC_{50}). DPPH is a free radical that can interact with compounds that can donate hydrogen atoms and can be used to measure the antioxidant activity of a nanoparticle preparation. This method has advantages because it is easy, fast, and sensitive for testing antioxidant activity[17].

Based on this study, the nanoparticles loaded jackfruit leaf extract which had the highest percent encapsulation efficiency, namely formula 2, were tested for antioxidant activity by measuring the IC_{50} of each test solution, which can be seen in Table 3. The IC_{50} value of nanoparticles loaded jackfruit leaf extract was 11.56 μg/ml±0,134 smaller than that of jackfruit leaf extract. This shows that the vesicle system of nanoparticles derived from chitosan polymer and sodium alginate can provide protection against jackfruit leaf extract which has flavonoid components that can act as antioxidants so that by making a nanoparticle system it can increase the stability of flavonoids in jackfruit leaf extract and can increase activity. antioxidants. With the development of a delivery system in the form of nanoparticles loaded with jackfruit leaf extract, it has the potential to maintain the stability of the active substance and can improve the delivery system of the active substance to its destination so that its effectiveness can increase.

4. CONCLUSION

Based on research that has been done that there is an effect of the amount of calcium chloride on the percent efficiency of encapsulation. The particle size, polydispersity index, zeta potential of the best formula showed good characterization for the nanoparticle system. The XRD pattern of nanoparticles loaded jackfruit leaf extract showed that an amorphous form was formed which could be more easily absorbed and easily dissolved in the form of nanoparticles. The nanoparticle system of jackfruit leaf extract showed an increase in antioxidant activity measured by the DPPH method when compared to jackfruit leaf extract.

ACKNOWLEDGMENT

An expression of the gratitude are towards to Universitas Sriwijaya for Kompetitif Research Grant Sains and Technology, Integrated Basic Laboratory FMIPA of Universitas Sriwijaya.

REFERENCES

[1] Ayumi D, Sumaiyah S, Masfria M. Pembatan dan Karakterisasi Nanopartikel Ekstrak Etanol Daun Ekor Naga (*Rhaphidophora pinnata*) Menggunakan Metode Gelasi Ioni. Talenta Coference Series: Tropical Medicine (TM). 2018:1(3): 29-33

[2] Pal SL, JPK Manna, GP Mohanta, Manavalan R. Nanoparticle an Overview of Preparation and Characterization. Journal Applied Pharmacy Science. 2011:1(6): 228-234.

[3] Untari B, Wijaya DP, Mardiyanto, Herlina, Anggraeni V, Firana A. Physical Interaction of Chitosan-Alginate Entrapping Extract of Papaya Leaf and Formation of Submicron Particles Dosage Form. Science and Technology Indonesia. 2019:4: 64-69.

[4] Mohanraj VJ, Chen Y. Nanopartcles: A Review. Tropical Journal of Pharmaceutical Research. 2006:5(1): 37-47

[5] Mardiyanto, Solihah I, Jaya TG. The Chitosan-Sodium Alginate Submicro Particles Loading Herbal of Ethanolic Extract of Leaves *Senna alata* L for Curing of Bacterial Infection on Skin. Science and Technology Indonesia. 2020:5: 85-89

[6] Masalova O, Kulikouskaya V, Shutava T, Agabekov V. Alginate and Chitosan Gel Nanoparticles for Efficient Protein Entrapment. Physycs Procedia. 2013:40: 69-75

[7] He J, Li R, Sun X, Wang W, Hu J, Xie H, Yin H. Effect of Calcium Alginate Submicroparticles on Sedd Germination and Seedling Growth of Wheat (*Triricum aestivum* L.). Polymers. 2018:10

[8] Sapana PA, Paraag SG, Shiravastav A, Pankaj S. Iontropic gelation: A promising Crosslinking Technique for Hydrogels. Journal of Nanotechnology. 2013:2(1): 234-238

[9] Loizzo MR, Tundis R, Chandrika UG, Abeysekera AM, Menichini F, Frega NG. Antioxidant and Antibacterial Activities on Foodborne Phatogens of *Artocarpus heterophyllus* Lam. (Moraceae) Leaves Extracts. Journal of Food Science. 2010; 75(5): 291-295.

[10] Shirvastava J. Comparative Analysis of Total Quercetin Content in Aqueous and Ethanolic Extract of *Artocarpus heterophyllus* by Liquid Chromatography. International Journal of Green and Herbal Chemistry. 2015:4(4): 501-507.

[11] Aluani D, Tzankova V, Burdina MK, Yordanov Y, Nikolova E, Odzhakov F, et al. Evaluation of Biocampatibility and antioxidant Efficiency of Chitosan-Algiante Nanoparticles Loaded with Quercetin. International Journal of Biological Macromolecules. 2017.

[12] Loizzo MR, Menichini F, Conforti F, Tundis R, Bonesi M, Saab AM, Statti GA, de Cindio B, Houghton PJ, Frega NG. Chemical analysis, antioxidant, antiinflamatory and anticholinesterase activities of *Origanum chren bergii* Boiss and *Origanum syriacum* L. Food Chemical. 2009. 117:174-180.

[13] Hermanto D, Mudasir M, Siswanta D, Kuswanti B. Synthesis of Alginate-Chitosan Polyelectrolyte Complex (PEC) Membrane and its Physical-Mechanical Properties. Jurnal Kimia Sains dan Aplikasi. 2019; 22(1):11-16

[14] Lindfors L, Skantze P. Amorphous Drug Nanosuspension Particle Dissolution and Crystal Growth, Langmir. 2007: 23(98); 66-74.

[15] Berne BJ, Pecora R. Dynamic light scattering: With application to chemistry Biology and Physic Dover Publication. 2000.

[16] Nimesh S, Chandra R, Gupta N. Advances In Nanomedicine For The Delivery of Therapeutic Nucleic Acids. Kent: Elsivier Secience. 2017.

[17] Koleva II, TA Van Beek. JPH Linnsen. A de Groot, LN Evstatieva Screening of plant exytact for antioxidant activity: A Comparative study on tree testing methods. Phytochemical Analysis. 2010; 1391:8-1

ICCSCP Atlantis Press Journal

ORIGINALITY REPORT

9%	4%	2%	6%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Sriwijaya University Student Paper	6%
2	Jun Nakamura, Nozomi Komiya. "Exploring Features of the Unconscious in Quality Inspection in Manufacturing", Journal of Robotics, Networking and Artificial Life, 2023 Publication	1%
3	jrssem.publikasiindonesia.id Internet Source	1%
4	1library.net Internet Source	1%
5	lppm.unram.ac.id Internet Source	1%
6	ftp.academicjournals.org Internet Source	1%

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		