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RESEARCH ARTICLE

Purple sweet potato leaf extract (*Ipomoea batatas* L.) Antin-3 variety on polyphenols content, antioxidant activity and in vitro penetration test

Damaranie Dipahayu[1], Hilya Nur Imtihani[2]

[1] Department of Pharmaceutical Technology, Republic of Indonesia Defence University, Indonesia
[2] Department of Pharmaceutical Technology, Hangtuah University, Indonesia

Keywords
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Correspondence
Damaranie Dipahayu
Department of Pharmaceutical Technology
Republic Indonesia Defence University
Indonesia
dipahayudamaranie@gmail.com

Abstract

Background: Nano capsules of extract of purple sweet potato leaves (Ipomoea batatas L.) Antin-3 variety were formulated to protect polyphenols, which are unstable to external environmental influences, and to enhance penetration into skin layers. **Objective:** This research investigates the effect of nano-capsulation on polyphenol content, antioxidant activity, and in vitro penetration. **Method:** Nano capsules were prepared by ionic gelation; the IC50 value was determined using the DPPH method, and a penetration test was conducted using a Franz diffusion cell. **Result:** Polyphenols of Antin-3 leaf extract nano capsule were 51.96 ± 11.74 mg GAE/gram. After the ultrasonic process, the concentration of polyphenols in the nano capsule was 190.94 ± 1.16 mg GAE/gram, indicating that the polyphenols in the nano capsule were 138.98 ± 12.78 mg GAE/gram. The entrapment efficiency value was 71.67%, and the loading capacity was 13.99%. The IC_{50} was 267.28 ppm (equivalent to 1/6 of pure vitamin C and ½ of Antin-3 leaf extract). A nano capsule of Antin-3 leaf extract penetrated across a membrane 1.7 times more easily than Antin-3 leaf extract. **Conclusion:** The research concludes that polyphenols in nano capsules show no detectable in vitro antioxidant activity via the DPPH method. Further testing is needed to assess the antioxidant activity using the Franz diffusion cell method.

Introduction

UV A and UV B radiation can induce the formation of free radicals in the skin, leading to DNA mutagenesis and cell damage, which ultimately results in cutaneous keratitis (Colina *et al.,* 2020). Skin needs an antioxidant that can protect it from UV radiation. There are two secondary metabolites found in plants, including flavonoids and polyphenols. Leaf extracts of purple sweet potatoes (*Ipomoea batatas* (L.)) variety Antin-3 had a total flavonoid content of 4.83% and a total polyphenol content of 16.98%. The leaf extract of Antin-3 also had an IC50 value of 47.99 ppm. Therefore, it can be used as a natural source of antioxidants (Dipahayu & Kusumo, 2020). Polyphenols are frequently unstable in response to surrounding environmental parameters, such as air and the pH value of the environment (Cefali, 2019). Cefali's research suggests that the chitosan nanoparticle formula can protect the plant's sensitive metabolites, such as polyphenols, thereby enhancing its antioxidant activity (Cefali, 2019).

From the previous research of Damaranie and Kusumo, they formulate nano-capsules of leaf extract Antin-3 using extract: chitosan: NaTPP with a ratio of 1:10:1, and the result shows that the suspensions of leaf extract Antin-3 nano-capsule has a long time to aggregate with one another, but it does have a relatively large size of 734,36 nm (Dipahayu & Kusumo Gondo, 2021). The nano capsule was formulated with a 1:5:1 ratio to maximise the protection of polyphenols and also to enhance skin penetration. Based on the above backgrounds, the researcher has reformulated

the nano capsule of Antin-3 leaf extract with a 1:5:1 ratio. The efficacy of the nano capsule formulation will be proven by its entrapment efficiency and loading capacity (Liang *et al*., 2018; Romanhole *et al*., 2020). On the other hand, this study also measured the antioxidant activity and the per cent penetration of that formula.

Methods

Material

Antin-3 leaf (BALITKABI), Chitosan, NaTPP, Glasial Acetic acid (Q-rec), Ethanol 96% (Rajawali, pharmaceutical grade, Surabaya, Indonesia), Aquadest (Rajawali, pharmaceutical grade, Surabaya, Indonesia), Gallic acid PA (Merck), Methanol (Merck), DPPH (Merck), Follin Ciocalteu, Na2C03, abdomen skin of white mice preparation (PNF).

Antin-3 leaf extract nanoencapsulation formulation

Nano capsules of an Antin-3 leaf extract formulation were prepared using the ionic gelation method. Ionic gelation method starts from adding the NaTPP solution (0.1%) to a mixture of chitosan solution (0.2%) and Antin-3 leaf extract solution (2%) with a ratio of NaTPP: chitosan: extract was 1:5:1. The NaTPP solution dripping rate was 0.1 mL/10 seconds, the process was also stirred with a stirring speed of 1400 rpm for 1 hour (Kurniasari & Atun, 2017) and (Katouzian & Jafari, 2016)

Polyphenols content, entrapment efficiency (EE) and loading efficiency (LE)

Polyphenols content

The polyphenol content assay of nano-capsule Antin-3 leaf extract was determined using the Folin-Ciocalteu method, with gallic acid as the standard (Jeong *et al*., 2004). A nano capsule of Antin-3 leaf extract, approximately 1 mL, is added to 0.2 mL of Folin-Ciocalteu reagent (50%), then mixed using a vortex for 3 minutes. This solution was added with 0.2 mL of 7.5% Na2CO3. Then restore to the dark chamber for 30 minutes. The absorbance was identified by UV-VIS spectrophotometry at 656 nm. The results were expressed as gallic acid equivalents in milligrams per kilogram of extract. The calibration curve was prepared in the same way using gallic acid as standard (Jeong *et al.,* 2004).

Entrapment efficiency (EE) and loading efficiency (LE)

Antitin-3 leaf extract nano capsules, approximately 10 mg, were added to 3 mL of ethanol, and the suspension was ultrasonicated using an ultrasonic cleaner (Kunshan Ultrasonic Instruments, KQ2200DB) for 4 hours to completely release antitin-3 from the purple sweet potato leaf extract. The supernatant was collected by centrifugation (8000 rpm for 10 min) and then measured using UV-vis spectroscopy (Shimadzu, UV-2550) to determine the concentration of antitin-3 polyphenols of purple sweet potato leaf extract in the supernatant. Measurement of polyphenol content was carried out using gallic acid as a standard (Liang *et al*, 2018).

LE and EE were calculated using the following:

The equation for measuring LE and EE:

$$EE\ (\%)\ = \frac{M}{Mo}\ x\ 100\ \%$$

$$LE\ (\%)\ = \frac{M}{W}\ x\ 100\ \%$$

Where M is the mass of purple sweet potato leaf extract antitin-3 in nanoencapsulation of purple sweet potato leaf extract antitin-3, M0 is the initial mass of nanoencapsulation of purple sweet potato leaf extract antitin-3, and W is the total amount of purple sweet potato leaf extract antitin-3 used for nanoencapsulation (Liang *et al.,* 2018)

Antioxidant activity

Antioxidant activity assay using the DPPH method was calculated by the percentage of DPPH reduction (as a radical substance) in the extract sample with a UV-Vis spectrophotometer instrument, with the following formula:

$$\%\ DPPH\ reduction\ = \frac{Control\ abs\ -\ samples\ abs}{Control\ abs}\ x\ 100\ \%$$

Furthermore, the IC_{50} value is determined, which is obtained from the linear regression equation of DPPH reduction against several concentration ranges of sample solutions (Kusumo & Dipahayu, 2022).

Franz diffusion test (percent penetration)

The Franz diffusion test was an in vitro instrument using the abdominal skin of white mice. Each of the nano-capsule Antin-3 leaf extract and Antin-3 leaf extract cream was applied approximately 1g to the mouse abdominal skin as the donor compartment membrane. Phosphate buffer at pH 7.4 was used as the receptor fluid in the Franz Diffusion Cell apparatus. The temperature used during the test was 37°C ± 1 °C. The media fluid flowed through the bottom of the skin membrane at a speed of 50 rpm. Sampling was carried out at minutes 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120, where 1 mL of sample was taken from the receptor

compartment using a syringe and immediately replaced with 1 mL of medium solution. The samples taken were then measured for absorbance using UV-Visible spectrophotometry (Mulyana *et al*., 2016)

Results

Nano capsule Antin-3 leaves extract formula, Polyphenols content, LE% and EE%

The powder of Antin-3 leaf extract nano capsule was 13.26 grams. There were two total phenolic assays before and after ultrasonic treatment. Total polyphenol of the sample before ultrasonication showed 51.96 ± 11.74 mg GAE/gram of extract, and the second test, after ultrasonication, showed 190.94 ± 1.16 mg GAE/gram of extract. That means the first was showing that the nano-capsule system was perfectly formed, then the chitosan-NaTPP nano-capsules were damaged by ultrasonics, and the polyphenols could be released from the capsule. The trapped polyphenols were 138.98 ± 12.78 mg GAE/gram of extract, the Efficiency entrapment that can be calculated was 72,79 % [(138 98 mg/ 190,94 mg) x 100 %], and the loading efficiency was 13,89 % [(138,98 mg/1000mg)x 100 %] (Figure1).

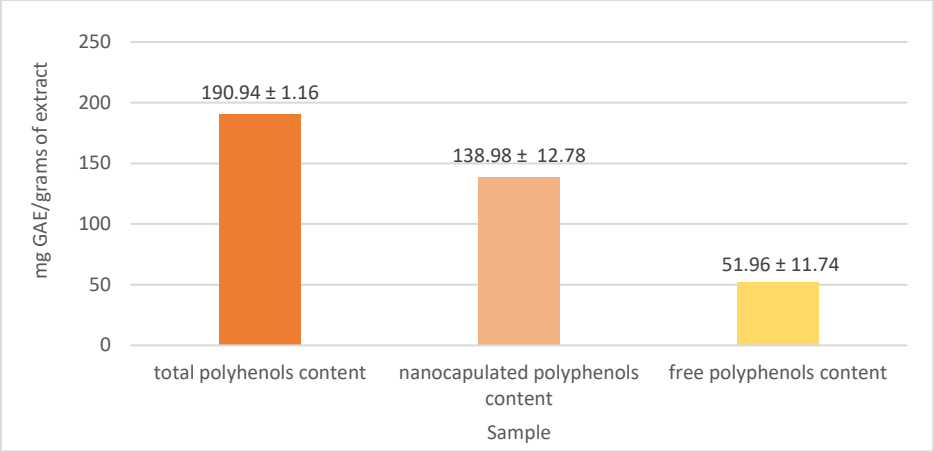

Figure 1: Polyphenol content of nano capsule leaf extract of Antin-3

Antioxidant capacity

Polyphenol compounds in the sample were the substances that were responsible for the antioxidant properties. In the DPPH method, polyphenols can scavenge the DPPH. The IC 50 value of nano capsules is almost ½ times higher or ½ times the antioxidant capacity than the extract form and 1/6 times the antioxidant capacity than pure vitamin C (Figure 2).

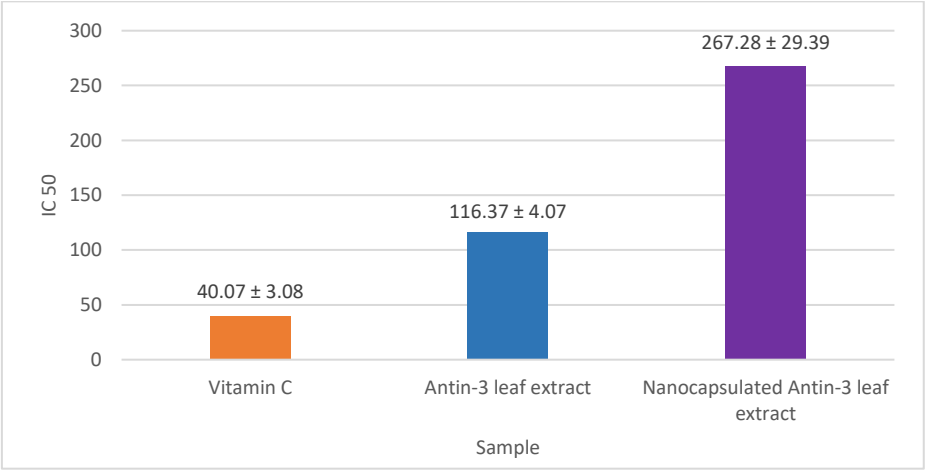

Figure 2: Antioxidant activity of samples

Penetration test

Antin-3 leaves extract nano capsules have the ability to penetrate the membrane of the white rat's stomach skin (in vitro) 1.7 times more effectively than the extract form (Figure 3).

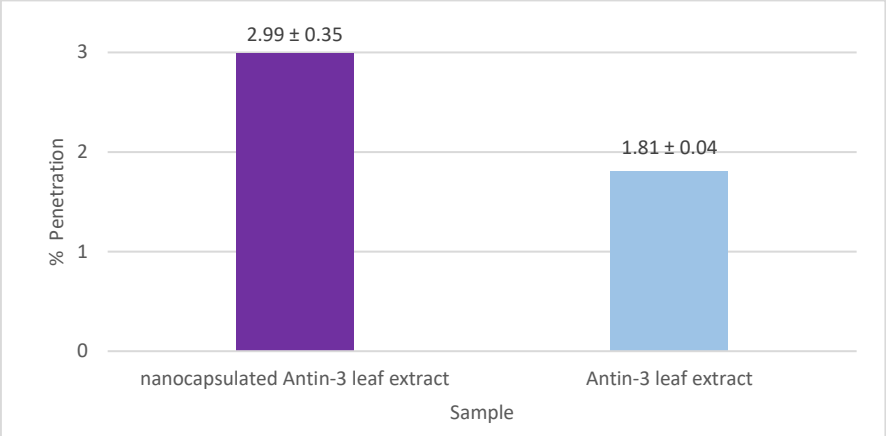

Figure 3: Penetration activity of samples

Discussion

The efficiency entrapment is influenced by the nano-capsule formulation method. In this study, the polymer dispersion method, also known as the ionic gelation method, was employed, which is based on the interaction between oppositely charged macromolecules. Chitosan is a long-chain polymer composed of glucosamine monomers (2-amino-2-deoxy-D-glucose). Chitosan is a positively charged polyelectrolyte that interacts with NaTPP, a negatively charged polyelectrolyte. The cross-linking bonds that form will strengthen the mechanical strength of the particles (Park & Yeo, 2007; Mohammed *et al*., 2017). The ratio of the concentration of the components, the formulation method and the pH of the nanocapsule system can affect the success and strength of the ionic gelation process formed. The advantage of the ionic gelation method is the high load capacity; however, the particle size produced has a relatively wide range of 200-1000 nm (Katouzian & Jafari, 2016).

The Antin-3 leaf extract nano capsule has the lowest potency antioxidant compared to the extract form and pure vitamin C, because 70% of the polyphenols are trapped in the chitosan-NaTPP nano capsules, so that they cannot reduce DPPH. Based on previous antioxidant activity test research by Kusumo and Dipahayu (2022), data has been obtained that the IC 50 value for the Antin-3 leaf extract nano capsule suspension sample with a ratio of NaTPP: chitosan: Antin-3 leaf extract = 1:10:1 is 48.67 ppm, this antioxidant potential value is 5 times higher than the Antin-3 leaf extract nano capsule sample with a ratio of 1:5:1. This can be because chitosan has effectiveness as an antioxidant. Chitosan has amino groups and two hydroxyl groups that can reduce free radicals; the suspension sample allows for the optimal effect of chitosan in reducing DPPH to be observed. The powder form of Antin-3 leaf extract nano capsules minimises the effectiveness of chitosan as an antioxidant (Abd El-Hack *et al*., 2020).

Nano capsules are a combination of delivery systems that can simultaneously increase skin penetration due to the outer layer of the capsule, which is a combination of water and polymer. This combination is capable of inducing local skin hydration and facilitating the delivery of active substances through the skin layer (Osorio-Blanco *et al*., 2020). The ionic gelation method enables particle size reduction, facilitating easier penetration of active substances. The formed nano capsules are expected to release polyphenols when they penetrate the membrane so that they can deliver polyphenols into deeper cells and provide antioxidant protection (Osorio-Blanco *et al*., 2020), this can be proven from the results of the calculation of the percentage of penetration where the nano capsule form is 1.7 times more penetrating the membrane than the extract form.

Conclusion

The nanoencapsulation formulation of Antin-3 leaf extract has been proven to encapsulate polyphenols in chitosan- NaTPP quite well, as evidenced by an

entrapment efficiency value of 70%, which means that only 30% of the polyphenols from the extract are outside the nano capsules. This results in only 30% of the polyphenols being able to reduce DPPH (in vitro test), so that the IC50 value becomes 1/6 of that of pure vitamin C. The Antin-3 leaf extract nano capsule penetrates the mouse skin membrane (Franz diffusion cell method) 1.7 times more effectively than the extract form. To prove the delivery system of that nano capsule through the membrane, this study needs to be continued as an antioxidant activity assay from the receptor compartment on the Franz diffusion cell (after membrane penetration).

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