Pharmacy Education (2022) 22(2) 151 - 155
https://doi.org/10.46542/pe.2022.222.151155

IAI SPECIAL EDITION

RESEARCH ARTICLE

In vitro anti-ageing activity of ethanol extract of Cantigi (*Vaccinium varingiaefolium Blume Miq*.) leaf and the extract loaded gelatin nanoparticles

Kosasih Kosasih, I Wayan Redja, Yunahara Farida
Fakultas Farmasi, Universitas Pancasila, Jakarta, Indonesia

Keywords
Anti-ageing
Cantigi
Extract
In vitro
Nanoparticle

Correspondence
Kosasih Kosasih
Fakultas Farmasi
Universitas Pancasila
Jakarta
Indonesia
kos_qs1@yahoo.com

Abstract

Background: Empirically, Cantigi leaves (*Vaccinium varingiaefolium* Blume Miq.) have been used for beauty and health, but their activity has not been studied much. **Objective:** To produce an ethanolic extract of Cantigi leaves, nanoparticles of the ethanolic extract and to compare their antioxidant, anticollagenase, and antityrosinase activities. **Methods:** Dry powders of young Cantigi leaves were macerated gradually with hexane, ethyl acetate, and ethanol. Dried extracts were evaluated for specific and non-specific parameters, made into nanoparticles by desolvation using gelatin as polymer and glutaraldehyde as a crosslinker, and then characterized. Antioxidant, anticollagenase, and antityrosinase activities of the extracts and nanoparticles (IC_{50}) were measured and compared. **Results:** The ethanol extract of Cantigi leaves met specific and non-specific parameters. Nanoparticle: particle size of 307.90±2.54 nm, polydispersity index of 0.271±0.01, zeta potential 12.80±0.44 mV, entrapment efficiency of 43.29±3.97%, and spherical. IC_{50} of anticollagenase, antioxidant, and antityrosinase for extracts 104.18±0.33, 18.87±0.36, 196.88±20.28 ppm; and for nanoparticles 124.40±0.39, 30.14±0.33, and 53.55±27.07 ppm, respectively. **Conclusion:** In general, the nanoparticle activity was lower than the extract activity except for the antityrosinase activity. The slow release of the extract from the nanoparticles is thought to be the cause of the decrease in activity.

Introduction

Cantigi (*Vaccinium varingiaefolium* Blume Miq.) is one of the endemic plants in Indonesia, growing well near crater volcanoes, and spreading in the archipelago (Sholikhah *et al.*, 2015). The previous report showed that the ethanol extract of young red leaves of Cantigi has very strong antioxidants (Kosasih *et al.*, 2016). Empirically, the leaves are used for salad and skin health (Maya, 2020). Antioxidant activity may lead to anti-ageing activity, as the red color indicates anthocyanin and flavonoid presence. In addition, Cantigi belongs to the same genus as Bilberry (*Vaccinium myrtillus*), which has been studied and marketed worldwide (Pires *et al.*, 2020).

Nanoparticles are a big part of the material. They, especially in the cosmetics industry, have provided great attention because of their significant physicochemical properties. The industries have used them for about 30 years (Fytianos *et al.*, 2020). The European Union defines a nanomaterial in cosmetics as a material that is insoluble or bio-persistent and intentionally manufactured with one or more external dimensions or an internal structure, on the size of 1 - 100 nm (Regulation (EC) No. 1223, 2009).

As a natural, biocompatible, biodegradable, and multifunctional biopolymer, gelatin has been used widely for encapsulating bioactive molecules in food, pharmaceutical, cosmetic, and medical applications. Gelatin nanoparticles provide more benefits compared to conventional release (Foox & Zilberman, 2015). Because of very little information and studies on the anti-ageing properties of leaf extract and leaf extract loaded nanoparticles of Cantigi, this study was conducted to compare their antioxidant, anticollagenase, and antityrosinase activities.

Material and methods

Chemicals and reagents

Cantigi leaves were obtained from Mount Tangkuban Parahu and identified at Universitas Indonesia, Jakarta. All chemicals and reagents used were analytical grades.

Preparation of extracts and nanoparticles

Dry powders of young Cantigi leaves were macerated (powder:solvent = 1:10, room temperature, 48 hours) gradually with hexane, ethyl acetate, and ethanol (Kosasih *et al.*, 2016). Dried extracts were evaluated for specific and non-specific parameters (Syukri *et al.*, 2020). The extracts were made into nanoparticles by desolvation method with gelatin as polymer and glutaraldehyde as a crosslinker and then characterized for particle size, polydispersity index, zeta potential, functional groups (FTIR), morphology, and percentage efficiency entrapment (Khan, 2014). The anti-ageing activities of the extracts and nanoparticles (as IC_{50}) were evaluated and compared using methods as described below.

Antioxidant assay

The assay was determined using the DPPH method (Desmiaty *et al.,* 2019) with slight modifications. A DPPH methanolic solution of 0.4 mM was prepared. 1 mL of the solution was added to each extract solution of 5-25 ppm concentrations; then the volume was set to 5 mL with ethanol addition. After mixing, the solution was kept for 30 minutes in a dark place, then measured at 517 nm. IC_{50} in ppm was calculated using a graph of extract concentration versus percent inhibition. IC_{50} of gelatin nanoparticles and vitamin C (as the control) were determined using the same method. Each experiment was conducted thrice, and the IC_{50} value was stated as mean ± SD.

Anti-tyrosinase assay

The assay was performed based on the method (Moon *et al.*, 2010) with slight modifications. The reaction was done with phosphate buffer (0.1 M, pH 6.7), 5 mM L-DOPA, and mushroom tyrosinase solution (310 units/mL) at 37°C in Nunc 96 well microtitre plate. Before adding the substrate, the mixture was incubated for 15 min. The absorbance change of dopachrome was measured at 490 nm using a microplate spectrophotometer (Versamac). Kojic acid (as the control) and gelatin nanoparticles were determined using the same method. The assay was performed thrice. The antityrosinase activity was calculated using this formula:

Antityrosinase activity (%) = (OD blank – OD extract) x 100 / OD blank

where OD blank and OD extract were the optical densities without and with the presence of extract or gelatin nanoparticle, respectively.

Dimethylsulfoxide (DMSO) was used as a blank, kojic acid as a standard inhibitor for tyrosinase, and phosphate buffer as a color control test (instead of the enzyme tyrosinase). Percent inhibitions of tyrosinase were expressed as a percentage of inhibition of tyrosinase activity.

Anti-collagenase assay

The assay was slightly modified based on the report by Nurrochmad and the authors in 2018. A 30 µL sample was diluted with 60 µL tricine buffer (50 mM, pH 7.5), 10 µL collagenase enzyme was added to each well, then the mixture was incubated at 37°C for 15 min. The substrate (FALGPA) was added to each well; the absorbance was measured at 335 nm using a microplate reader. Water was used as the negative control, and EGCG 250 µM (0.114 mg/mL) was used as the positive control.

Results

Table I shows the specific and non-specific data of the ethanol extracts. The data was a part of extract standardisation. In general, all evaluated parameters met the standards (Ditjen POM RI, 2000). Table II (A) shows the formula of Cantigi extract-loaded gelatin nanoparticles used in the study based on previous reports (Khan, 2014; Kosasih *et al.*, 2019). The study in 2019 used ethanol extract as the active ingredient. The desolvation method was used to manufacture nanoparticles because it is simple, fast, and inexpensive (Khan, 2014). In addition, Table II (B) shows that the water content and polydispersity index were within the ranges. Meanwhile, the particles size did not comply with the range of 1-100 nm as defined by the EU but complied with the other definition of 1 - 1000 (Foox & Zilberman, 2015). The solubility data of nanoparticles was important to know and could be used for further analysis or development, such as entrapment efficiency evaluation and formulation. Good zeta potential was about ±30 mV indicating the stability of nanoparticles. Nevertheless, the stability could be achieved by any means, such as adding Pluronic F68. In this study, entrapment efficiency of the extract was 43.29%; meaning 1 g nanoparticles contained only 432.9 mg of extract. Improvements in formulas and processes should be conducted to increase entrapment efficiency. Moreover, Table II (C) shows that the band

at about 1466 cm^{-1} indicated the formation of aldimine linkage (CH=N) as the reaction of the aldehyde group of glutaraldehyde (crosslinker) with the amino group of gelatin. This is consistent with the previous report (Khan, 2014).

Table I: Specific and non-specific parameters of the Cantigi extracts

		Description
Cantigi leaf weight (g)		500.20
Amount of viscous extract (g)		82.46
DER-native		6.07
Yield (%)		16.49
Organoleptic	Color	Reddish green
	Odor	Cantigi specific
	Form	Viscous
pH		3.23±0.10
Water content (%)		7.38±0.38
Solubility	Ethanol 96%	1:100 (sparingly soluble)
	Methanol	1:100 (sparingly soluble)
	Acetone	1:100 (sparingly soluble)
	Purified water	1:100 (sparingly soluble)
	DMSO	1:10 (freely soluble)
Total ash content (%)		7.75±0.82
Acid insoluble ash content (%)		1.41+0.10
Water-soluble content (%)		14.67±0.45
Ethanol soluble content (%)		12.83±0.89
Microbiology	Total plate count	Negative
	Yeast mold count	
Heavy metal	Pb (lead)	< 0,357 ppm
	Cd (Cadmium)	< 0,002 ppm

Table III shows the anti-ageing properties of Cantigi extracts and nanoparticles expressed in anti collagenase, antioxidant, and antityrosinase activity. Antioxidant activity was very strong, while anti collagenase and antityrosinase activity were weak. Changing extracts into nanoparticles generally cause the decreased activity of anti collagenase and antioxidants except antityrosinase. Generally, extract entrapped in nanoparticles takes time for release, and this might cause the activity to decrease. Figure 1 shows the morphology of Cantigi extract-loaded gelatin nanoparticles enlarged 3000 times using SEM. It was spherical and consistent with the previous report (Kosasih *et al.*, 2019). This is the first report on the anti-ageing activities of Cantigi leaf extracts and gelatin nanoparticles loaded with the extracts.

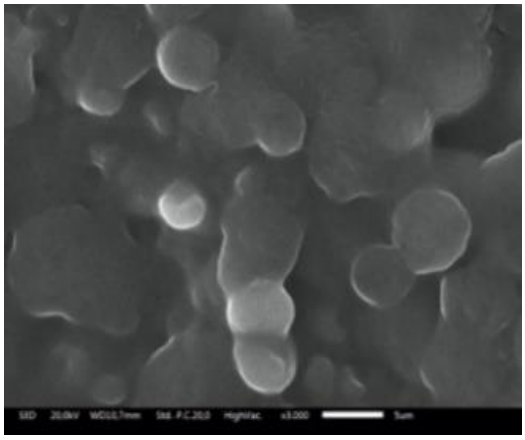

Figure 1: Morphology of the nanoparticles

Table II: Formula of Cantigi extract-loaded gelatin nanoparticles (A), characteristics of the nanoparticles (B), and FTIR data of gelatin, extract, and the nanoparticles (C)

Formula of Cantigi extract-loaded gelatin nanoparticles (A)		Characteristics of the nanoparticles (B)	
Ingredient	**Amount**	**Parameter**	**Description**
		Organoleptic:	
		Color	Light brown
Gelatin	200 mg	Odor	Cantigi specific
Purified water	25 mL	Form	Powder
Cantigi extract	100 mg	Solubility in Solvent:	
Ethanol 96%	15 mL	Ethanol 96%	1:10 (freely soluble)
DMSO	10 mL	Methanol	1:10 (freely soluble)
Purified water	5 mL	Acetone	1:100 (sparing. sol.)
Pluronic F68	300 mg	DMSO	1:10 (freely soluble)
HCl 0.1N	q.s	Water	1:10 (freely soluble)
Acetone	40 mL	Water content (%)	8.17±0.70
Glutaraldehyde	0.3 mL	Particle size (nm)	307,87±2.54
		Polydispersity index	0,271±0,01
		Zeta potential (mV)	12,80 ± 0,44
		Entrapment efficiency (%)	43.29±3.97

Functional group	Wave-number (cm^{-1})	FTIR data of gelatin, extract, and the nanoparticles (C) Wave-number (cm^{-1})		
		Gelatin	Extract	Nanoparticle
-OH	3150-3650	3280	3315	3314
N-H	3000-3500	3074	-	-
C-H stretching (aliphatic)	2700-3000	2960	2926	2882
C=N	1400-1690	1449	1443	1466
C=O	1650-1900	1625	1603	1635
CH (Hydrocarbon)	1300-1475	1334	1342	1342
C=C	650-1000	918	912	961

Table III: Anti-ageing activities of Cantigi extracts and nanoparticles

Activity	IC_{50} (ppm) Extracts	Nanoparticles
Anticollagenase	104±0.55	124.47±0.26
Antioxidant	18.87±0.36	30.14±0.33
Antityrosinase	196,88±20,28	53,55±27,07

Conclusion

The study results show that the nanoparticle anti-ageing activity was lower than the extract anti-ageing activity except for the antityrosinase activity. The slow release of the extract from the nanoparticles was thought to be the cause of the decrease in activity.

Acknowledgements

The authors thank Alfred, Chaterine, Hanny, Husnah, Indah, Rahma, Septian for helping in this research. This paper has been presented orally at Rakernas & PIT IAI Virtual 2021 "*The Opportunities of Pharmacists Digital Services in Pandemic Recovery*", Jakarta, Indonesia.

Conflicts of interest

The authors state that there is no conflict of interest.

References

Desmiaty, Y., Saputri, F.C., Hanafi, M., Prastiwi, R., Elya, B. (2020). Anti-elastase, anti-tyrosinase, and antioxidant of Rubus fraxinifolius stem methanolic extract. *Pharmacognosy Journal,* **12**(2), 271-275. https://doi.org/10.5530/pj.2020.12.42

Ditjen POM RI. (2000). Parameter Standar Umum Ekstrak Tumbuhan Obat. Cetakan Pertama. Depkes RI, Jakarta

Foox, M., Zilberman, M. (2015). Drug delivery from gelatin-based systems, *Expert Opinion on Drug Delivery*, **12**(9), 1547-1563, https://doi.org/10.1517/17425247.2015.1037272

Fytianos, G., Rahdar, A., Kyzas, G.Z. (2020). Nanomaterials in cosmetics: Recent updates. *Nanomaterials*, **10**, 979. https://doi.org/10.3390/nano10050979

Khan, S.A. (2014). Gelatin nanoparticles as potential nanocarriers for macromolecular drugs. Dissertation. Phillips University Marburg, Marburg

Kosasih, K., Yulyana, A., Winarno, H. (2016). Karakterisasi ekstrak daun Cantigi (Vaccinium varingiaefolium (Blume) Miq.). *Jurnal Sains dan Kesehatan*, **1**(5), 276-283. https://doi.org/10.25026/jskv1i5.50

Kosasih, K., Sumaryono, W., Supriyono, A., Mudhakir, D., Cristian, Y.E., Debora, R. (2019). Effects of gelatin and glutaraldehyde concentrations on characteristics of cantigi (Vaccinium varingiaefolium Miq.) extract loaded gelatin nanoparticles as antioxidant. The 2019 International Joint Symposium, Universitas Airlangga, Surabaya

Maya I. (2020). Cantigi ungu, anti penuaan dini dan antioksidan alami (online). Available at: https://farmasetika.com/ 2020/ 01/29/cantigi-ungu-anti-penuaan-dini-dan antioksidan-alami/

Moon, J-Y., Yim, E-Y., Song, G., Lee, N.H., Hyun, C-G. (2010). Screening of elastase and tyrosinase inhibitory activity from Jeju Island plants. *Eurasian Journal of Bioscience*, **53**(March), 41-53. https://doi.org/10.5053/ejobios.2010.4.0.6

Nurrochmad, A., Wirasti, Dirman, A., Lukitaningsih, E., Rahmawati, A., Fakhrudin, N. (2018). Effects of antioxidant, anticollagenase, antielastase, antityrosinase of the extract and fraction from Turbinaria decurrens Bory. *Indonesian Journal of Pharmacy*, **29**(4), 188–197. https://doi.org/10.14499/indonesianj pharm29iss4pp188

Pires, T.C.S.P., Caleja, C., Santos-Buelga, C., Barros, L., Ferreira, I.C.F.R. (2020). Vaccinium myrtillus L. fuits as a novel source of phenolic compounds with health benefits and industrial applications - A review. Curren *Pharmaceutical Design*, **26**(16), 1917-1928. https://doi.org/ 10.2174/1381612826666200317132507

Regulation (EC) No. 1223/2009 (online). Available at: https://ec.europa.eu/growth/sectors/ cosmetics/ products/ nanom Taterials_en (accessed on 8 August 2021)

Sholikhah, A., Dian, F.A., Listyorini, D. (2017). Anatomy and morphological study of Mentigi Gunung (Vaccinium varingiaefolium (Blume) Miq.) in area of Mount Batok, Indonesia. *KnE Life Science*, 36-45. https://doi.org/ 10. 18502/ kls.v3i4.685

Syukri, Y., Purwati, R., Hazami, N., Tahmid, H.A., Fitria, A. (2020). Standardization of specific and non-specific parameters of Propolis extract as raw material for herbal product. *Eksakta*, **1**(1), 36-43. https://doi.org/10.20885/EKSAKTA.vol1.iss1.art6