Antioxidant Activity Test Combination Of Cantigi Leaf Extract (Vaccinium varingiaefolium) And Nail Henna Leaf Extract (Lawsonia inermis Linn)

Mokhamad mahroji[1*], Shelly Taurhesia[2], Dian Ratih Laksmitawati[3]

[1,2,3] Master of Pharmaceutical Sciences, Faculty of Pharmacy, Pancasila University
Jl. Raya Lenteng Agung No. 56-80, RT 1/ RW 3, Srengseng Sawah,
Jagakarsa 12630, South Jakarta, Indonesia
[*] Corresponding author:
Email : ojierasyidi11@gmail.com

Abstract.
Traditionally, cantigi leaves (Vaccinium varingiaefolium) are used to heal wounds, swelling, burning, pain, ulcers, and function as analgesic, anti-inflammatory and anti-inflammatory. inflammation, and treat fine wrinkles . henna leaves (Lawsonia inermis Linn) which contains glycosides, steroids, phytosterols, saponins, tannins, and flavonoids which are reported to have benefits for treating boils, scabies, headaches, back pain, and can accelerate hair growth . Cantigi leaves and fruit are reported to contain anthocyanin compounds that function as antioxidants . while the antioxidants contained in cantigi leaves are Beta.-mono-olein , Hexadecanoic acid, and methyl ester . The flavonoids contained in henna leaves also have the potential as antioxidants. We are often exposed to oxidation both produced from within the body or from outside such as pollution, cigarette smoke, UV radiation and food. Antioxidants will neutralize these free radicals so they do not have the ability to steal electrons from cells and DNA. Cantigi leaves and henna leaves were macerated by kinetic maceration method with 70% ethanol as solvent. Antioxidants were measured using the DPPH method, the single extract of cantigi gave an IC 50 value of 119.23 ± 4.41 µg/mL , while the extract of a single nail henna gave an IC 50 value of 38.38 ± µg/mL. The combination of cantigi leaf extract and nail henna leaf extract with a ratio of 1:1; 2:1 and 1:2 gave very strong IC50 values, namely 33.84 µg/mL; 40.40 µg/mL and 29.85 µg/mL, respectively. It was concluded that the combination of cantigi leaf extract and nail henna leaf extract with a ratio of 1:2 gave the best IC50 value of 29.85 µg/mL.
Keywords : *Vaccinium varingiaefolium; Lawsonia inermis Linn; Antioxidant; DPPH*

I. INTRODUCTION

Free radicals in the body can cause degenerative diseases and premature aging. Free radicals are formed due to the presence of oxidants either from within the body itself or from outside such as environmental conditions where pollution, cigarette smoke, UV rays, and food. Antioxidants are needed to prevent cell or DNA damage caused by free radicals. To maintain antioxidant activity, it is necessary to use a combination of antioxidants. As can be explained in the example of vitamin C which is able to maintain the activity of vitamin E as an antioxidant by regenerating tocopherol from tocoperoxyl radicals. Vitamin E which is a very strong antioxidant will bind to free radicals, then turn into inactive tocoperoxyl radicals . Previous research concluded that Vitamins C and E will not work optimally against antioxidants.

Vitamins C maintains the amount of vitamin E in cells by reforming vitamin E radicals to a reduced form and vitamin E inhibits macromolecular and DNA damage caused by oxidation of vitamin C.

(1) Indonesia is rich in natural resources, including biological natural resources. Chemical compounds in plants such as anthocyanins, flavonoids, and polyphenol groups are reported to have antioxidant activity, including cantigi leaves (Vaccinium varingiaefolium) (12) and henna leaves (Lawsonia inermis Linn). (3) Christian (2021) research on the antioxidant power of 96% ethanol extract of cantigi leaves using the DPPH method obtained an IC50 value of 16.84 µg/mL (2) , while Husni (2018) investigated the antioxidant power of 70% ethanol extract of henna leaves using the FRAP method, obtained an antioxidant power of 2.82 mmol Fe (II)/100 g (3). For this reason, in this study, the antioxidant activity of the single extract of cantigi leaves and the single extract of henna nails leaves will be measured using the DPPH method. The IC50 values obtained from each extract will be compared against the combination of

cantigi leaf extract and nail henna leaf extract. The ratio of the combination of cantigi leaf extract and henna leaf extract used was 1:1; 1:2 and 2:1. Antioxidant activity can be further classified into very strong, strong, moderate and weak as shown in Table 1 below.

Table 1. Classification of the strength of antioxidant activity (5)

Concentration IC_{50} (µg/mL)	Activity classification Antioxidant
< 50	Very strong
50 – 100	Strong
100 – 250	Currently
250 – 500	Weak
> 500	Not active

II. METHODS

This study used an experimental method in selecting a combination of cantigi leaf extract (Vaccinium varingiaefolium) and nail henna leaf extract (Lawsonia inermis Linn) which has very strong antioxidant power, as indicated by the best IC50 value of a ratio of 1:1; 2:1 and 1:2.

Place and time of research

The research was conducted at the Faculty of Pharmacy, Pancasila University and UPTD. PPMHP Banten Province. The research period starts from June 2021 to December 2021, with letter number 523/PPMHP-046.A/V/2021.

Research Sample

The materials used in this study were cantigi leaves obtained from Mount Papandayan, Garut Regency and henna leaves obtained from Padarincang Serang Regency.

Research Tools

Analytical Balance, Homogenizer, maceration apparatus, rotary evaporator, Uv-Vis Spectrophotometer, pH–meter, Brookfield Viscometer, Memmert Oven, pyrex test tube, test tube rack .

Extract Making

Using the kinetic maceration method, with 70% ethanol as solvent and the extraction was carried out several times. Then the extract was concentrated with an evaporator at a temperature of 400C and a thick extract was obtained. (8)

Quality Test of cantigi leaf extract (CLE) and nail henna leaf extract (NHLE)

After obtaining a thick extract, a quality test is carried out, which includes:

- Specific parameters (organoleptic, water soluble extract content, ethanol soluble extract content) (5,6)
- Non-specific parameters (drying shrinkage determination of moisture content, determination of total ash content, acid insoluble ash content, determination of residual solvent) (5,6)
- Test for microbial and heavy metal contamination (7).
- Photochemical screening of each extract was carried out to see the secondary metabolites contained in each extract (15,10).

Antioxidant Activity Test by DPPH method

Weighed a number of single thick extracts of cantigi leaves and extracts of henna nails leaves and dissolved in methanol p.a to obtain a concentration of 1000 µg/mL, for CLE a concentration series of 0 was made; 10.47; 20.94; 41.88; 83.75; and 167.50, as well as for NHLE 0; 4.28; 8.56; 17.13; 34.25 and 68.50 then for the combined extract, a number of thick extracts of cantigi leaves were weighed and the extract of henna nails was added in a ratio of 1:1; 1:2 and 2:1, and homogenize. The combination of these extracts was dissolved in methanol p.a until a concentration of 1000 µg/mL was obtained, from which a series of concentrations was made from 0; 3.13; 6.25; 12.50; 25 and 50 µg/mL. A total of 2 mL of each test solution was added 2 mL of 0.002% DPPH. homogenized then incubated at room temperature for 30 minutes, measure the absorbance at a wavelength of 515 nm with a UV-Vis spectrophotometer. (11).

III. RESULTS AND DISCUSSION

Extract Making

The yield of CLE and NHLE from kinetic maceration using 70% ethanol solvent which was thenconcentrated with a rotary evaporator were 25.60% and 28.01%, respectively. As shown in Table 2

Table 2. Extraction Results and Yield Results

Sample	Simplicity (g)	Solvent (%)	Thick Extract (g)	DER- *native* (%)	Yield (%)
Cantigi Leaf Extract (CLE)	281.6	Ethanol 70%	72.1	3,906	25,60
Nail Girlfriend Leaf Extract (NHLE)	534	Ethanol 70%	149.6	3,570	28.01

Quality Test of Cantigi Leaf Extract and Nail Henna Leaf Extract

The results of the quality test of the two extracts can be seen in Tables 3, 4, 5 and 6, indicating that the specific and non-specific parameters met the requirements. And the results of the microbial and heavy metal contamination tests also meet the requirements in accordance with the 2017 FHI regulations(6,7)

Table 3. Specific Parameter Quality Test Results

Specific parameters	Cantigi leaf extract (CLE)	Nail henna leaf extract (NHLE)
	Organoleptic	
Shape	Thick	Thick
Color	Dark Chocolate	Dark Chocolate
Smell	Special Extract	Special Extract
Water soluble juice content	81.40%	58.49%
Ethanol soluble extract content	12.47%	12.58%

Table 4. Non-specific parameter quality test results

Non-specific parameters	CLE	NHLE	Condition (Depkes RI 2017)
Drying shrink	10.07%	13.0%	≥10%
Water content	5.41%	5.71%	≤10%
Total ash content	6,41%	2,91%	≤10%
Acid insoluble ash content	0,36%	0,82%	≤10%
Residual solvent	Not detected	0,2%	≤1%

Table 5. Results of determination of microbial contamination

Parameters of Microbial Contamination	CLE	NHLE	Condition (BPOM RI 2014)
ALT	<10 cfu/g	<10 cfu/g	10^6 cfu/g
AKK	<10 cfu/g	<10 cfu/g	10^4 cfu/g

Table 6. Results of determination of metal contamination

Metal Contamination Parameter	CLE mg/kg	NHLE mg/kg	Condition (BPOM RI 2014)
Mercury (Hg)	0.0002	0.0001	1 mg/kg
Lead (Pb)	0.0019	Not detected	20 mg/kg
Cadmium (Cd)	0.0017	0.0017	5 mg/kg
Manganese (Mn)	0.7329	0.7258	5 mg/kg

Table 7. Results of phytochemical screening analysis

Test Parameters	Reactor	Observation	Results CLE	Results NHLE
Alkaloids	Chloroform,+ H_2SO_4 Mayer Dragendroff	White precipitate Yellow precipitate	+ +	+ +
Flavonoids	Powder Mg + H_2O + HCl	Yellow orange	+	+
Saponins	H_2O + HCl	Embossed foam	+	+
Tannins	H_2O+ $FeCl_3$	Greenish black	+	+
Steroids/Triterpenoids	CH3COOH + H2SO4 _	greenish	+ Steroids / - Triterpenoids	- Steroids / - Triterpenoids
Phenol	$FeCl_3$	Dark blue	+	+

Phytochemical screening aims to provide an overview of the initial group of compounds found in the studied plants (15, 10). The phytochemical screening from CLE showed positive results for secondary metabolites of alkaloids, flavonoids, saponins, tannins, steroids and phenols. While NHLE showed positive results for secondary metabolites of alkaloids, flavonoids, saponins, tannins and phenols.

Antioxidant Test using the DPPH. method

Ascorbic acid is a raw material for comparison with the category of having very strong antioxidant activity. The $IC_{50\ value}$ is an indicator of the interpretation of the test results of antioxidant activity using the DPPH method. (14) Ascorbic acid has a polyhydroxy group that will increase antioxidant activity. (4) The results of the duplo measurement for antioxidants with DPPH obtained the $IC_{50\ value}$ of ascorbic acid of 1.81±0.02 g/mL as shown in graph 1, which shows a very strong antioxidant power.

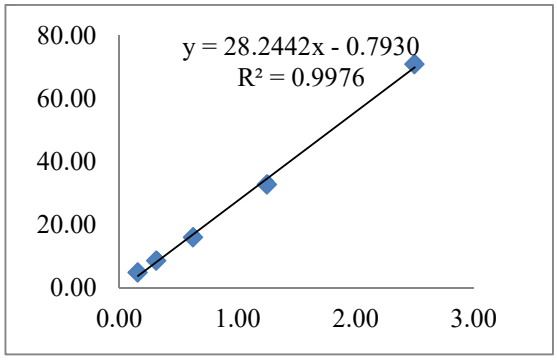

Fig 1. Measurement of antioxidant ascorbic acid using DPPH

Ascorbic acid is a raw material for comparison with the category of having very strong antioxidant activity. The $IC_{50\ value}$ is an indicator of the interpretation of the test results of antioxidant activity using the DPPH method. (14) Ascorbic acid has a polyhydroxy group that will increase antioxidant activity. (4)

Table 8. Antioxidant Test Results Single Extract of Cantigi Leaves

Concentration (g/mL)	1 . measurement		2 . measurement	
	Absorbance 515	% inhibition	Absorbance 515	% inhibition
Blank	0.2556		0.2556	
10.47	0.2551	0.20	0.2508	1.88
20.94	0.2333	8.72	0.2333	8.72
41.88	0.1941	24.06	0.1942	24.02
83.75	0.1570	38.58	0.1454	43.11
167.50	0.0775	69.68	0.0775	69.68
IC50 value	**116.11**		**122.34**	

From the analysis results obtained IC50 levels $_{of}$ ascorbic acid are 1.81±0.02 g/mL , this shows a very strong interpretation of the results, as shown in graph 1. Table 8 shows the antioxidant activity test of a single extract of cantigi leaves obtained IC levels $_{50}$ was 119.23±4.41 g/mL which showed moderate

antioxidant activity. Nail henna leaf extract gave an IC $_{50\ value}$ of 38.38 ± 1.67 g/mL , which is a very strong antioxidant, as shown in Table 9.

Table 9. Antioxidant Test Results Singel Extract of Nail Henna Leaf

Concentration (g/mL)	1 . measurement		2 . measurement	
	Absorbance 515	% inhibition	Absorbance 515	% inhibition
Blank	0.2956		0.2834	
4.28	0.2699	8.69	0.2727	3.78
8.56	0.2515	14.92	0.2541	10.34
17.13	0.2161	26.89	0.2184	22.94
34.25	0.1549	47.60	0.1543	45.55
68.50	0.0589	80.07	0.0559	80.28
IC50 value	39.55		37.20	

The IC50 value of the combined antioxidant activity test results of CLE and NHLE with a ratio of 1:1, 2:1, 1:2, can be seen in Table 10.

Table 10. Test Results of Combination Extract Antioxidant Activity

Sample	Test Repeat	IC Rated $_{50}$	X ± SD IC50 (µg/mL)	Interpretation
1CLE:1NHLE	1 2	33.91 33.76	33.84±0.10	Very strong
2CLE:1NHLE	1 2	40.02 40.78	40,40±0.54	Very strong
1CLE:2NHLE	1 2	31.45 28.24	29.85±2.27	Very strong

The three mixtures of CLE and NHLE showed synergistic antioxidant activity , with IC50 values of $_{33.84}$ ±0.10 g/mL , 40.40±0.54 g/mL , and 29.85±2.27 g/ml, respectively. -consecutive for 1:1 combination ratio; 2:1 and 1:2 . Initially, a single extract of cantigi leaves only had moderate antioxidant activity. The antioxidant activity of CLE can be increased by combining it with NHLE and provides very strong antioxidant activity.

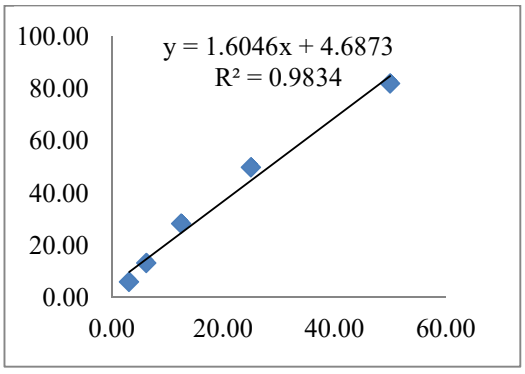

Fig 2. The results of the 1 : 2 combination antioxidant test from CLE and NHLE

Of the three comparisons of the combination of CLE and NHLE, a 2:1 ratio gives the best IC50 value of 29.85 g/mL as depicted in graph 2.

IV. CONCLUSION

Cantigi leaf extract has moderate antioxidant activity with an IC50 value of 119.23±4.41 µg/mL, nail henna leaf extract has very strong antioxidant activity with an IC50 value of 38.38±1.67 µg/mL . However, the IC50 value of cantigi leaf extract can be increased when combined with henna leaf extract, and the best result is the combination with a 1:2 ratio which provides very strong antioxidant activity with an IC50 value of 29.85 µg/mL..

V. ACKNOWLEDGEMENTS

This research article has been completed, the authors would like to thank the supervisors who have devoted their guidance and direction, and all who have helped in the research.

REFERENCES

[1] Andre O. Barel, Marc Paye, Howard I. Maibach. Handbook of cosmetic science and technology - 3rd ed. 2009. pages : 301-307

[2] Christian YE, Debora R. Effect of Gelatin and Glutaraldehyde Concentration on Characteristics of Gelatin Nanoparticles Containing Cantigi Extract (Vaccinium varingiaefolium Miq.) As Antioxidant Effect Of Gelatin And Glutaraldehyde Concentration On Characteristic Of Cantigi (Vaccinium Varingiaefolium Miq.) EXTRACT. 2021:1-7. doi:10.20473/jhpr.vol.4-issue.1.1-7. 3.

[3] Husni E, Suharti N, Pasella A, Atma T, Pharmacy F, Andalas U. Characterization of Simplicia and Leaf Extract of Girlfriend Nails (Lawsonia inermis Linn) and Determination of Total Phenolic Levels and Antioxidant Activity Test. 2018;5(1):12–6.

[4] Konda JP, Siampa JP, Tallei TE, Kepel BJ, Fatimawali F. Antioxidant activity of methanol extracts of Langsat (Lansium domesticum var. pubescens) and Duku (Lansium domesticum var. domesticum) seeds using the DPPH method. ***J Science.*** 2020;20(2):113.

[5] Ministry of Health of the Republic of Indonesia A. General Standard Parameters of Medicinal Plant Extracts. Jakarta: Bakti Husada; 2000.

[6] Ministry of Health of the Republic of Indonesia. Indonesian Herbal Pharmacopoeia Edition II . Jakarta.. 2017;213–8.

[7] Regulation of the Head of the Food and Drug Supervisory Agency Number 17 of 2014 concerning Amendments to the Regulation of the Head of BPOM Number HK.03.1.23.07.11.6662 of 2011 concerning Microbial Contamination Requirements And Heavy Metals In Cosmetics. Jakarta.;1–5.

[8] Rizky Amelia F. Determination of Tannin Types and Determination of Tannin Levels from Young Bungur Fruit (Lagerstroemia speciosa Pers.) Spectrophotometrically and Permanganometrically. . ***J Ilm Mhs Univ Surabaya.*** 2015;4(2):1.1 0 .

[9] Mailuhu M, Runtuwene MRJ, Koleangan HSJ. Phytochemical Screening and Antioxidant Activity of Soyogik Bark (Saurauia bracteosa DC.) Methanol Extract. ChemProg. 2017;10(1):1–6.

[10] Pukumuang W. Total phenolic contents, antibacterial and antioxidant activities of some Thai medicinal plant extracts. ***J Med Plants Res. 2012***;6(36):4953–60

[11] Sadiyah ER, Kodir RA. Preliminary Study of Anthocyanin Content in Purple Cantigi Fruit (V accinium V aringiaefolium (BL.) MIQ.) Y Which Functions As An Antioxidant Supplement. 2012;95–100.

[12] Sari AK, Ayati R. Determination of Antioxidant Activity of Ethanol Extract of Kaffir lime Leaf (Citrus hystrix D. C) by DPPH method (1,1-diphenyl-2-picrylhydrazyl). ***J Curr Pharm Sci***. 2018;1(2):69–74.

[13] Sari DM, Anwar E, Nurjanah, Arifianti AE. Antioxidant and tyrosinase inhibitor activities of ethanol extracts of brown seaweed (Turbinaria conoides) as lightening ingredient. ***Pharmacogn J.*** 2019;11(2):379–82.

[14] Simare E. Phytochemical Screening Ethanol Extract of Itchy Leaves (Laportea decumana (Roxb.) Wedd). Pharmacy. 2014;11(01):undefined.