Journal of Tropical Pharmacy and Chemistry

Journal homepage: https://jtpc.farmasi.unmul.ac.id

Antioxidant Activity Test of Effervescent Granules Morinda citrifolia L Leaf Extract with DPPH Free Radical Absorption Method

Muthmainah Tuldjanah[1,*], Niluh Puspita Dewi[2], Dian Rahmawati[1]

[1]Diploma Programme Pharmacy, STIFA Pelita Mas, Palu, Central Sulawesi, Indonesia
[2]Bachelor Degree Pharmacy, STIFA Pelita Mas, Palu, Central Sulawesi, Indonesia
*Corresponding author: muthmainah.tuldjannah@gmail.com

Abstract

Morinda citrifolia. L leaf is a plant that has the potential as an antioxidant due to the presence of compounds in the form of flavonoids and other phenolic compounds that can function as natural antioxidants. In connection with this, research was carried out to *Morinda citrifolia*. L formulate leaf extract in the form of effervescent granules. *Morinda citrifolia* leaf extract was macerated with ethanol 96% as solvent. Phytochemical screening showsthat the extract contains flavonoids, tannins, saponins and alkaloids. The extract was formulated with various concentrations of F1 (20%), F2 (25%), F3 (4%). Determination of antioxidant activity using the DPPH free radical immersion method. The results showed that the concentration in the granules influenced antioxidant activity with IC50 values of 109.05 ppm, 101.33 ppm and 73.28 ppm, respectively.

Keywords: Antioxidant activity, DPPH, Effervescent granules, *Morinda citrifolia*. L leaf

Submitted: 17 October 2020 **Accepted**: 26 June 2021 **DOI**: https://doi.org/10.25026/jtpc.v5i4.285

1 Introduction

The *Morinda citrifolia* L plant is a plant that can grow easily in tropical areas such as Indonesia and Malaysia. *Morinda citrifolia* L one tan safe drug that has enough potential to be developed as it contains several substances that had to include: antioxidants, alkaloids, anthraquinone, flavonoids, tannins, saponins and vitamin C. In this regard of *Morinda citrifolia* L fruit, containing antioxidant which is quite high, namely 324.70 mg/100 g in *Morinda citrifolia* L juice [3].

Morinda citrifolia L leaves contain 5 flavonol glycosides, namely: quercetin-3-O-β-D glucopyranosides; kaemperol-3-O-α-L-ramnopirosil-(1→6)-β-Dg lucopyranosides; quercetin-3-O-α-L-ramnopyrosil (1→6)-β-D-glucopyranoside; quercetin-3-O-β-D-glucopyranosil-(1→2)-[α-L ramnopyranosil-(1→6)-β-D-glucopyranoside; and kaemperol-3 -

O-β-D-glucopyranosil (1→2)-[αL-ramnopiranosil-(1→6)β-D-galakopyranoside. Compounds lives of polyphenols such as compounds of flavonoids (including flavonols) capable of inhibiting autooxidation through the mechanism of arrest radical (radical scavenging) with how to donate an electron from unpaired electrons in free radicals sehing ga amounts of free radicals is reduced [1]

Antioxidants are natural substances or compounds that can protect body cells from damage and aging caused by free radicals. The human body can neutralize free radicals with endogenous antioxidants, but if the number of free radicals is excessive, the ability to neutralize them decreases. The body needs exogenous antioxidants that can help protect the body from free radical attack by reducing the negative impact of free radicals. [1]. The antioxidant activity test was used and accepted by the researchers as an anticancer clue. However, the antioxidant activity does not fully describe it so that other information is needed such as antimutogenicity, anti-inflammatory, and anti-proliferative [2,4].

Effervescent granules are granules or crude powder that contain medicinal elements in a dry mixture. This preparation usually consists of sodium carbonate, carbonic acid and tatric acid. When added with water, the acid and carbon dioxide will react and free carbon dioxide to produce foam [6,7]. The advantages of effervescent granules compared to ordinary powder drinks are the ability to produce carbon dioxide gas which gives a fresh taste, covers the bitter taste of medicinal ingredients, accelerates the absorption of medicinal substances in the stomach, and is practical in storage and transportation compared to liquid drinks [8].

The aim of this research is to formulate the effervescent granule of *Morinda citrifolia* L leaf extract and to test the antioxidant activity of the effervescent granules produced by the DPPH free radical reduction method.

2 Materials and Methods

2.1 Phytochemical screening test

Phytochemical screening is carried out to detect the presence of secondary metabolites based on their group and as initial information to determine which chemical compounds have biological activity from a plant in the form of simplicia or extract. Tests were carried out on a class of alkaloid compounds, flavonoids, saponins, and tannins which were carried out qualitatively by color reaction or precipitation [5].

2.2 Making Morinda Citrifolia. L Leaf Extract

Morinda citrifolia L leaves are obtained in the Taweili area, northern Palu sub-district, Palu city. *Morinda citrifolia* L leaves are taken as much as 2 kg, sorted wet , washed and dried to air. Then chopped to obtain the *Morinda citrifolia* L leaf simplicia that is ready for extraction. 400 g of simplicia was macerated using 96 % ethanol. The sample was filtered and the filtrate was evaporated using a rotary evaporator at a temperature of 60 °C until 118 g of viscous extract was obtained.

2.3 Formulation of Morinda citrifolia L Leaf Extract Effervescent Granules

Table 1. Granull effervescent formula of *Morinda citrifolia* L leaf extract

Material	Uses	Formula (%)			
		F1	F2	F3	F4
Morinda citrifolia L Leaf Extract	Active substance	-	20	25	30
Sodium bicarbonate	Alkaline component	25	25	25	25
Citric acid	Acid component	11	11	11	11
Tartaric acid	Acid component	14	14	14	14
PVP	Binder	2	2	2	2
Na CMC	*Suspending agent*	1	1	1	1

2.4 Effervescent Granule Manufacturing

The preparation of effervescent granules was carried out using the wet granulation method. The steps taken are as follows:

1. Each crystal-shaped material such as citric acid and tartic acid is first pollinated by grinding. Then sieve with a sieve No. 60, then weigh.
2. The thick *Morinda citrifolia* L Leaf Extract is then mixed with sieved sodium bicarbonate and sodium cmc (mixture 1).
3. Then add the citric acid and tartic acid which have been mashed (Mixture 2).

4. Grind until homogeneous then add PVP which has been dissolved in alcohol. Dry in oven at 50 °C until completely dry. After the mixture is dry, then sieve with a sieve No. 14

2.5 Antioxidant Activity

Antioxidant activity testing was carried out using the DPPH method. The DPPH concentration used in this study was 80 ppm at the wave length of 516 nm. Determination of the concentration series is done by making a stock solution of 1000 ppm and then a series of concentrations of 60, 80, 100, 120, 140, and 160 ppm are made. Then the absorbance was measured at a maximum wavelength of 516 nm. Replicated 3 times and determined the value of IC_{50}. DPPH radical scavenger activity (%) is calculated by equation 1.

$$\%Inhibition = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\% \qquad \text{(equation 1)}$$

Note:
Abs. Blank = 50 µM DPPH Absorbance
Abs. Sample = Absorbance of Test Sample

2.6 Analysis

The physical quality test data and the IC_{50} granule effervescent value data obtained were analyzed descriptively, by comparing the results obtained with the standard for determining antioxidant activity. Analysis of descriptive an analysis of the most basic to describe the state of the data in general. Data value of IC_{50} granule effervescent were obtained were analyzed also by using the test One way Anova analysis at significant level of 95%.

3 Results and Discussion

3.1 Phytochemical Screening

The results of the phytochemical screening of noni leaf extract (Morinda citrifolia L) are shown in Table 2.

Based on the results obtained in the phytochemical screening test, it showed that the extract of *Morinda citrifolia* L leaves contained flavonoids, tannin alkaloids and saponins. Phytochemical testing aims to determine the various kinds of chemical substances found in plants (MOH 1989).

3.2 Antioxidant activity of Granule effervescent

The antioxidant activity of *Morinda citrifolia* L leaves was tested using the DPPH method. Measurements were made using a spectrophotometer UV-Vis pad a maximum wavelength of 516 nm , in order to obtain the value of% inhibition of each formula. The IC_{50} value in each formula is determined using a linear regression equation from the curve of the relationship between sample concentration and percent inhibition with the equation Y = ax + b, sample concentration (ppm) as the axis (X) and the percentage value of inhibition as the axis (Y). Where the results of the IC_{50} value can be seen in the table 3, 4, 5, and 6.

Table 2. Phytochemical Screening Results of phytochemical *Morinda citrifolia* L leaf extract

No	Phytochemical test	Result
1	Flavonoid	+
2	Alkaloid	+
3	Tanin	+
4	Saponin	+

Table 3. IC_{50} values of F1 Effervescent Granules

Concetration (ppm)	Average	% inhibition	IC_{50} (ppm)
60	0,676	14,50	349.51
80	0,669	15,47	
100	0,583	26,25	
120	0,570	27,90	
140	0,563	28,78	
160	0,561	29,08	

Table 4. IC_{50} Value of F2 Effervescent Granule

Concetration (ppm)	Average	% inhibition	IC_{50} (ppm)
60	0,532	32,79	109,05
80	0,523	33,92	
100	0,444	43,87	
120	0,382	51,75	
140	0,265	66,46	
160	0,196	75,18	

Table 5. IC_{50} Value of F3 Effervescent Granule

Concetration (ppm)	Average	% inhibition	IC_{50} (ppm)
60	0,513	35,15	101,33
80	0,470	40,58	
100	0,395	50,02	
120	0,302	61,82	
140	0,205	74,08	
160	0,168	78,72	

Table 6. IC_{50} Value of F4 Effervescent Granule

Concetration (ppm)	Average	% inhibition	IC_{50} (ppm)
60	0,509	35.65	73,28
80	0,401	49,26	
100	0,309	60,94	
120	0,204	74,25	
140	0,186	76,53	
160	0,154	80,53	

Based on the linear regression equation, the relationship between the concentration of the extract and the percentage of inhibition, the IC_{50} values are 349.51, 109.69, 101.33 and 73.28 respectively, the comparison of the percentage of extracts in the granules shows the effect of antioxidants on the IC_{50} results on the activity test. antioxidants showed that the higher the percentage of the extract in the formula, the more it showed a higher IC_{50} value. This is presumably because the higher the concentration in the formula, the greater the content of secondary metebolite compounds contained in the formula.

The results of measuring the antioxidant activity of formula 1 show weak activity, formula 2 and 3 show moderate antioxidant formula and formula 4 shows strong antioxidant activity, this is according to the literature which states that the levels of antioxidant activity in the DPPH method consist of 4, namely IC_{50} <50 ppm is very strong, IC_{50} 50-100 ppm is strong, IC_{50} is 100-250 ppm is medium, and IC_{50} is 250-500 ppm is weak [3, 8] .

The result of statistical test of 1-way analysis of variance (one way ANOVA) shows that the IC_{50} ad value is a significant difference in formula 1 with the other 3 formulas. However, formulas 2 and 3 do not show a significant difference. Based on these results, the formula that has a good antioxidant activity is formula 4 because it shows the highest antioxidant activity and the statistical results show a difference with other formulas.

4 Conclusions

Based on the results of the research that has been obtained, it can be concluded that the secondary metabolites found in *Morinda citrifolia* L leaf extract in the phytochemical screening test are flavonoids, alkaloids, tannins and saponins. The antioxidant activity of F1 showed weak activity, namely 349.51, F2 and F3 showed moderate antioxidant activity, namely 109.09, 101.33, while formula 4 showed strong antioxidant activity, namely 73.28.

5 Acknowledgments

The author would like to thank DIKTI for fully funding this research through a Research Grant.

6 References

[1] Arief, S. 2007. Radikal Bebas. [Artikel] Ilmu Kesehatan Anak FK UNAIR.. RSU Dr. Soetomo, Surabaya.

[2] Siagian., Priska. 2012. Keajaiban Antioksidan. PT. Gramedia Pustaka Utama. Jakarta. Hal:1- 19.

[3] E. Syahruddin, H. Abbas, E. Purwati, dan Y. Heryandi. 2012. Aplikasi Mengkudu Sebagai Sumber Antioksidan Untuk Mengatasi Stress Ayam Broiler di Daerah Tropis. Jurnal Peternakan Indonesia Vol. 14 (3). Fakultas Peternakan Universitas Andalas Kampus Unand Limau Manis Padang.

[4] Lingga, L. 2012. The Healing Power of Antioxidant. PT Elex Media. Jakarta. 28-31, 69, 87-91, 109-111.

[5] Anonim. 2014 Farmakope Indonesia, Edisi V. Departemen Kesehatan Republik Indonesia. Jakarta. 53, 519, 601

[6] Ansel, H.C. Pengantar Bentuk Sediaan Farmasi. Diterjemahkan oleh Farida Ibrahim. 1989. Edisi IV. UI Press. Jakarta. 202-203, 214-2017. Dalam W, Sinta. 2014. Uji Aktivitas Antioksidan Granul Effervescent Ekstrak Kulit Buah Naga Merah (Hylocereus polyrhizus)

[7] Ganjar., Ibnu dan Rohman. 2012. Kimia Farmasi Analisis. Pustaka Pelajar. Yogyakarta. L: 220-262.

[8] Jannah, R. N., Fadraersada, J., Meylina, L., & Ramadhan, A. M. (2018). Formulasi Granul Antioksidan Ekstrak Etanol Daun Sirsak (Anonna muricata Linn.) Menggunakan Metode Granulasi Basah. *Proceeding of Mulawarman Pharmaceuticals Conferences*, *8*(1), 97-103. https://doi.org/10.25026/mpc.v8i1.310