Section A-Research paper

Isolation and Activity Test of Secondary Metabolites in Parijoto Fruit Methanol Extract (*Medinilla speciosa* Blume) as Antioxidant and Anticholestherol in Vitro

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ABSTRACT

Free radicals are associated with degenerative diseases such as cholesterol, the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, and premature aging. Long-term use of synthetic antioxidants has a toxic effect compared to natural antioxidants, so it is necessary to explore natural antioxidants to replace synthetic antioxidants, such as using plants containing flavonoids and phenols. It is known that the extract and methanol fraction of Parijoto fruit contained flavonoids, saponins, tannins, and glycosides. This study aimed to determine whether extracts, fractions and isolates of Parijoto fruit (Medinilla speciosa Blume) have antioxidant and anticholesterol activity in vitro. This study was carried out in several stages, namely extraction, fractionation, and isolation of parijoto fruit. The parijoto fruit underwent antioxidant activity test in vitro using the DPPH method and anticholesterol activity in vitro testing using the Lieberman-Burchard method. The collected data underwent normality and homogeneity tests. If the results are normal and homogeneous, One Way Anova parametric test is carried out, followed by post-hoc LSD. If the results obtained are not normal and not homogeneous, then a non-parametric test analysis is carried out, namely Kruskal Wallis and continued by Mann Whitney**.** The antioxidant and anticholesterol tests showed significant differences between each group. The 40.64 µg/ml methanol fraction group had the highest antioxidant activity while the lower methanol isolate had the highest anticholesterol atcivity of 94.27%. Methanol extract, methanol fraction, n-hexane fraction, upper methanol isolate, lower methanol isolate from Parijoto fruit had potential as antioxidants and anticholesterol in vitro.

KEYWORDS: Parijoto fruit isolate (Medinilla speciosa Blume), Antioxidant, Anticholesterol, DPPH method, Lieberman-Burchard method.

INTRODUCTION:

Free radicals are associated with degenerative diseases such as cholesterol, the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, and premature aging. Based on WHO data in 2008, 55% or 7.9 million deaths in Southeast Asia were caused by degenerative diseases[16]. Therefore, antioxidants are needed to inhibit free radicals. Low concentration antioxidants are significantly able to prevent diseases caused by free radicals[11]. Long-term use of synthetic antioxidants has a toxic effect compared to natural antioxidants such as allergies, asthma, nasal inflammation, headaches, to decreased consciousness. Thus, the search for natural antioxidants continues to be needed to replace synthetic antioxidants such as using plants.

According to Nishanthini (2012), plants containing secondary metabolites in the form of flavonoids and phenols have antioxidant and anticholesterol activity[17]. One of the Melastomacceae family species is Medinilla speciosa Blume or Parijoto fruit as a typical plant growing in the Colo, Kudus, Central Java[25]. The methanol extract and fraction of Parijoto fruit contains flavonoids, saponins, tannins, and glycosides[23]. The n-hexane extract of parijoto fruit contains terpenoids[18]. Meanwhile, the n-hexane fraction of parijoto fruit has anthocyanin compounds[14]. Antioxidant activity can be tested using the DPPH method. This method is fast, has high specificity, and is suitable for evaluating the radical scavenging activity of non-enzymatic antioxidants. To determine the most active anticholesterol activity, extracts, fractions, and isolates of Parijoto undergo the Lieberman-Burchard method. This method is used because of its high specificity in measuring steroid compounds in the form of cholesterol. In this study, the isolation of parijoto fruit was carried out because it is suspected that there are compounds that have the potential to have antioxidant and anticholesterol activities. This aimed to obtain pure compound isolates from an active fraction[24]. Therefore, this can be a development for the discovery of new drugs[6].

Purpose: To determine the in vitro antioxidant activity using DPPH method and in vitro anticholesterol activity using Lieberman-Burchard method of the extract, fraction, and isolate of parijoto fruit (Medinilla speciosa Blume).

Benefit: Provides scientific information related to extracts, fractions and isolates of parijoto fruit (Medinilla speciosa Blume) as antioxidants in vitro using the DPPH method and anticholesterol in vitro using the Lieberman-Burchard method.

Hypothesis: Extracts, fractions, and isolates of parijoto fruit (Medinilla speciosa Blume) have antioxidants in vitro using the DPPH method and anticholesterol in vitro using the Lieberman-Burchard method.

MATERIAL AND METHODS:

Chemicals and reagents:

The materials used were parijoto fruits from Kudus, methanol, n-hexane, distilled water, ethyl acetate, chloroform, cerium, concentrated sulfuric acid, silica, DPPH powder, cholesterol raw materials, Mg powder, quercetin, concentrated HCl, NaOH, H2SO4, FeCl3, glacial acetic acid, anhydrous acetic acid, and vitamin C.

Instrumentation:

The tools used were Uv-Vis spectrophotometry, sintered glass, water bath, rotary evaporator, gram and milligram electric scales (osuka), glassware (Pyrex), cup, micropipette, oven (memmert), Vortex mixer, chamber, and TLC sprayer.

Plant Determination

Plant determination was carried out by observing the morphology through the identification key showing the characteristics of the plant taxon. Identification and determination was carried out in the Biology Laboratory FMIPA Universitas Negeri Semarang.

Preparation of Parijoto Fruit Methanol Extract

Preparation of parijoto fruit methanol extract was started by the extraction process using the maceration method with methanol solvent ratio (1:10).

Fractination

The partition in the extract was carried out using methanol and n-hexane as solvent. 10 g of the extract was dissolved by adding 100 ml of methanol until the extract could be poured into a separating funnel. Then 100 ml of n-hexane was added into the separating funnel and shaken for 5 minutes while occasionally opening the faucet on the separating funnel so that the gas formed can be removed. The solution formed was allowed to stand for several minutes until the boundary between the methanol layer and the n-hexane layer could be seen. Furthermore, the partition was carried out in the same way until the n-hexane solvent became clear[27].

Isolation

Parijoto fruit isolates were prepared using the preparative TLC method on the stationary phase of silica gel 60 F254 plates with a size of 20 cm x 20 cm. The mobile phase of ethyl acetate and n-hexane had a ratio of 1:10. The elution results formed bands at each Rf value. Before carrying out readings under UV light at wavelengths of 254 nm and 366 nm, spraying was carried out with cerium sulfate for easy reading. The isolate obtained from preparative TLC was taken by scraping the bands on the silica gel plate and filtered by being dissolved using n-hexane and ethyl acetate solvents, then placed on a water bath at 50ºC.

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Antioxidant Activity Test

The samples used were extract, fraction, and isolate of parijoto fruit (Medinilla speciosa Blume). Each extract, fraction, and isolate was weighed up to 10 mg, then put into a 10 ml volumetric flask and diluted using methanol, then mixed to homogenize, so that a concentration of 1000 ppm was obtained as the mother liquor. Furthermore, the serial solution of each sample was prepared in concentrations of 20, 40, 60, 80, 100 ppm. 1 ml of sample at each concentration was taken and added 3 ml of 0.1 mM DPPH solution to be placed in a test tube, vortexed for 20 seconds, incubated for 30 minutes, and replicated the spectrophotometric readings 3 times[10].

Anticholesterol Activity Test

To determine the anticholesterol activity of 1000 ppm concentrate from methanol isolate and parijoto n-hexane, concentrations of 50, 75, 100, 125, and 150 ppm must be made. 5 ml of the mixture was taken, vortexed for 2 minutes, then added with anhydrous acetic acid and concentrated H2S04 of 2 ml and 0.1 ml respectively. For 15 minutes, the solution was allowed to stand until it turned green. The color results obtained were read on a UV-Vis spectrophotometer with a maximum wavelength. 5 ml of methanol and 5 ml of n-hexane were added with 2 ml of anhydrous acetic acid and 0.1 ml of concentrated H2SO4 as blanks. The negative control used 5 ml of cholesterol solution with a concentration of 100 ppm dissolved in methanol and n-hexane was added anhydrous acetic acid and concentrated H2S04 of 2 ml and 0.1 ml, respectively[2].

Data Analysis

Data on antioxidant and anticholesterol activity underwent statistical analysis. Data from each concentration and control series underwent normality test using Shapiro-Wilk and homogeneity test using Levene Test. If normal and homogeneous, the One Way ANOVA parametric test with a confidence level of $p<0.05$ would be carried out. If there is a significant difference, then it is continued by using post-hoc LSD. If it is not normal and not homogeneous, then the non-parametric Kruskal Wallis test and Mann Whitney would be carried out.

Results

The study was carried out in July 2021 – September 2021 at the Integrated Laboratory of the Pharmacy Study Program, Faculty of Medicine UNISSULA and Biology Laboratory UNNES. Antioxidant activity of vitamin C is presented in Table 1 and its curve is presented in Figure 1. Antioxidant activity of methanol extract, methanol fraction, and n-hexane fraction can be seen in Table 2 and its curve can be seen in Figure 2. IC_{50} of extracts and fractions compared to vitamin C is shown in Figure 3.

Table 1. Antioxidant activity of vitamin C

Sample	Cocentration (ppm) (x)	Average Absorbance	Average % Inhibition (y)	IC_{50} (µg/ml)
	5	0.7032	7.9581	
	10	0.6256	18.1152	
	15	0.5388	29.4633	23.32
Vitamin C	20	0.4466	41.5576	
	25	0.3522	53.8743	
	30	0.2551	66.5968	

Negative control absorbance = 0.7640

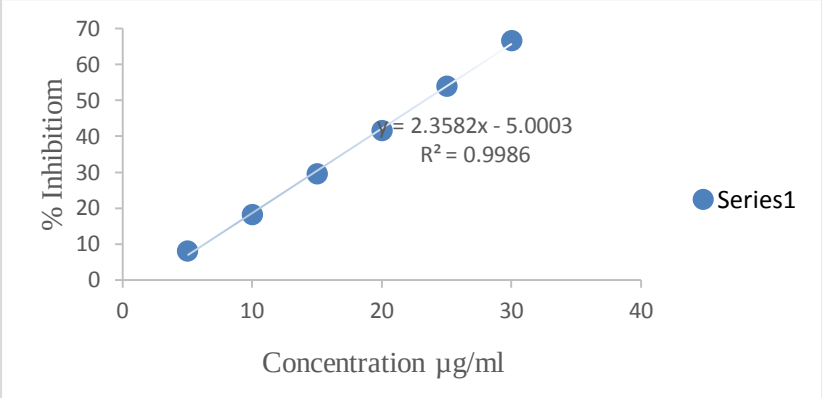

Figure 1. Antioxidant activity curve of vitamin C

Table 2. Antioxidant activity of methanol extract, methanol fraction, and n-hexane fraction

Sample	Concentration (µg/ml)	Average Absorbance	Average % Inhibition	Regression Equation	IC_{50} (µg/ml)
Methanol Extract	20	0.5167	32.3647	y = 0.7305x + 18.164 r = 0.9977	43.58
	40	0.3906	48.3508		
	60	0.3004	60.6806		
	80	0.1684	77.9581		
	100	0.0717	90.6097		
Methanol Fraction	20	0.4913	35.6937	y = 0.743x + 19.799 r = 0.9981	40.64
	40	0.3885	49.1492		
	60	0.2838	62.8664		
	80	0.1589	79.2277		
	100	0.0385	94.9520		
N-hexane fraction	20	0.6455	15.5061	y = 0.1122x + 13.036 r = 0.9848	329.44
	40	0.6306	17.4564		
	60	0.6131	19.7426		
	80	0.6009	21.3438		
	100	0.5747	24.7775		

Negative control absorbance = 0.7640

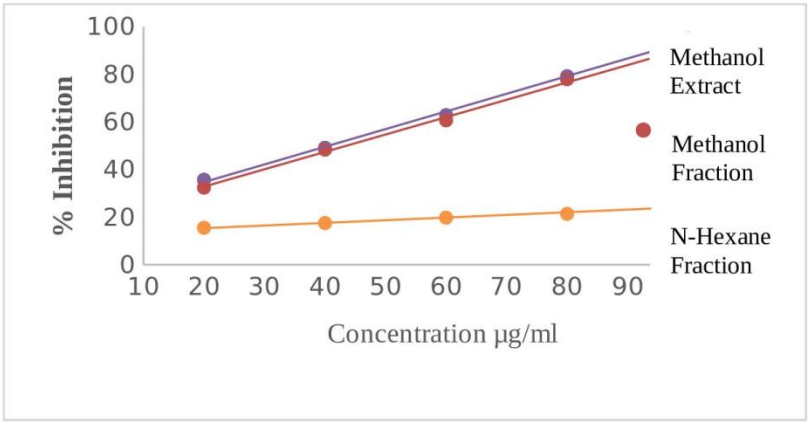

Figure 2. Antioxidant activity curve of methanol extract, methanol fraction, and n-hexane fraction

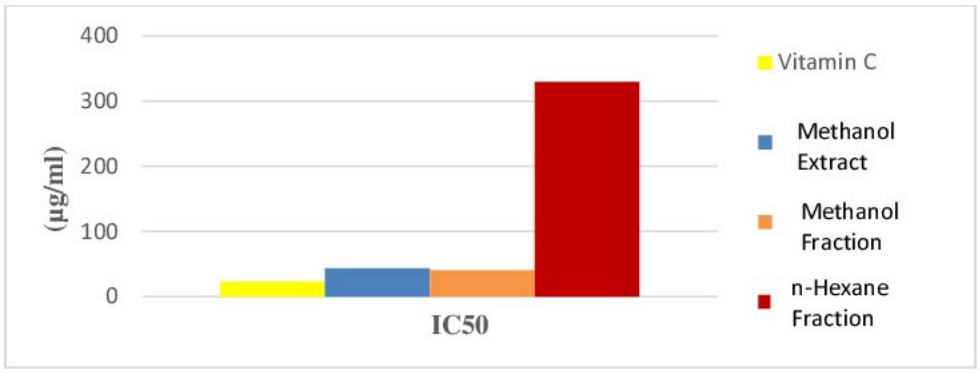

Figure 3. IC_{50} value of methanol extract, methanol fraction, n-hexane fraction, and Vitamin C

The decrease in cholesterol levels in the positive control, methanol extract, methanol fraction, and n-hexane fraction are presented in Table 3 and its curve can be seen in Figure 4.

Table 3. The decrease in cholesterol levels of the positive control, methanol extract, methanol fraction, and n-hexane fraction of Parijoto Fruit

Sample	Concentration (µg/ml)	Average Absorbance	Regression Equation	Cholesterol Level	Decrease
Positive Control (Simvastatin)	50	0,5922	y = 0,014x + 0,5193 r = 0,8884	8,26 ppm	53,11%
	75	0,5702		7,83 ppm	55,57%
	100	0,5676		7,78 ppm	55,86%
	125	0,5621		7,67 ppm	56,47%
	150	0,5337		7,11 ppm	59,64%
Methanol Extract	50	0,6134	y = 0,021x + 0,4874 r = 0,9701	8,68 ppm	50,74%
	75	0,5889		8,19 ppm	53,48%
	100	0,5771		7,96 ppm	54,79%
	125	0,5635		7,69 ppm	56,31%
	150	0,5323		7,08 ppm	59,80%
Methanol Fraction	50	0,8081	y = 0,0412x + 0,2528 r = 0,853	12,51 ppm	28,98%
	75	0,7898		12,15 ppm	31,02%
	100	0,6923		10,23 ppm	41,92%
	125	0,6872		10,13 ppm	42,49%
	150	0,6749		9,89 ppm	43,86%
N-hexane fraction	50	0,7831	y = 0,0256x + 0,2913 r = 0,9622	12,02 ppm	31,77%
	75	0,7540		11,44 ppm	35,02%
	100	0,7484		11,33 ppm	35,65%
	125	0,7189		10,75 ppm	38,95%
	150	0,6863		10,11 ppm	42,59%

Negative control absorbance = 1.0674

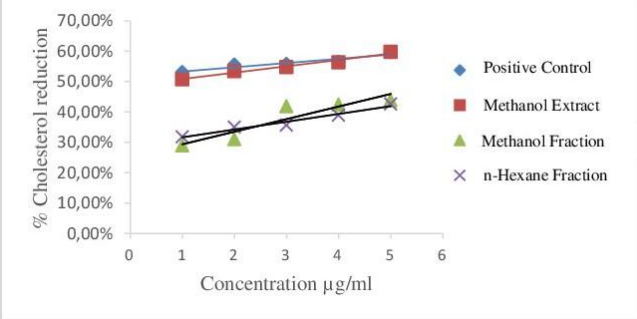

Figure 4. Anticholsterol Activity Curve of positive control, methanol extract, methanol fraction, and n-hexane fraction

Active Fraction Isolation Process

After testing the antioxidant and anticholesterol activity of the extract and fraction, the most active fraction was found in the methanol fraction, so the isolation process was continued on the methanol fraction. The isolation results of Parijoto fruit showed two stains, namely Rf 0.87 on the upper stain and Rf 0.50 on the lower stain. This indicates that the methanol fraction has two compounds that can be isolated from the methanol fraction as shown in Figure 5.

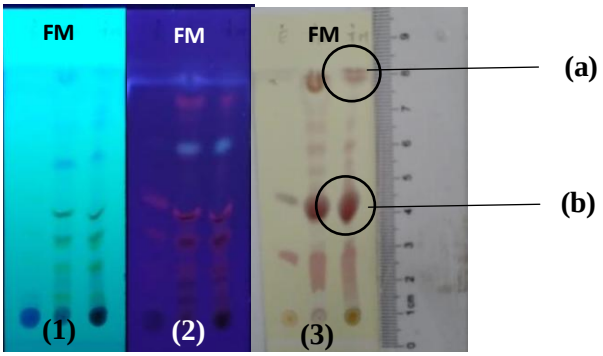

Figure 5. Upper Stain of Parijoto Fruit Methanol Fraction (a) and Lower Stain (b) with n-Hexane Eluent and Ethyl Acetate (5:1). (1) UV 254 nm, (2) UV 366 nm, (3) After being sprayed with cerium sulfate and heated

Table 4. Antioxidant activity of Upper and Lower Methanol Isolate

Sample	Concentration (µg/ml)	Average Absorbance	Average % Inhibition	Regression Equation	IC_{50} (µg/ml)
Upper Methanol Isolate	20	0.5705	25.3272	y = 0.623x + 12.355	
	40	0.4877	36.1562		
	60	0.3884	49.1623	r = 0.994	60.42
	80	0.2702	64.6335		
	100	0.2033	73.3813		
Lower Methanol Isolate	20	0.5316	30.4188	y = 0.735x + 13.157	
	40	0.4567	40.2181		
	60	0.3393	55.5890	r = 0.9924	50.12
	80	0.2133	72.0768		
	100	0.0917	87.9930		

Negative control absorbance = 0.7640

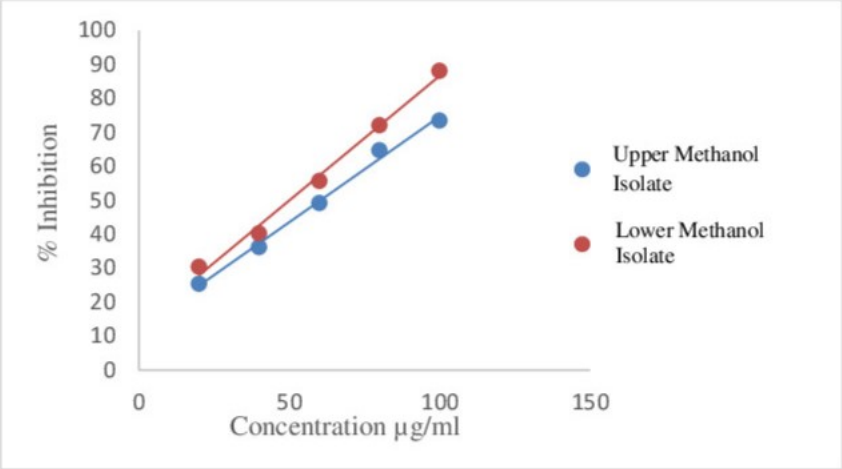

Figure 6. Antioxidant activity curve of Upper and Lower Methanol Isolate

Table 5. The decrease in cholesterol levels of Upper Methanol Isolate and Lower Methanol Isolate

Sampel	Concentration (µg/ml)	Average Absorbance	Regression Equation	Cholesterol Level	Decrease
Upper Methanol Isolate	50	0.6117	y = 0.051x + 0.4668	8.64 ppm	50.93%
	75	0.5556		7.54 ppm	57.20%
	100	0.5113		6.67 ppm	62.15%
	125	0.4491	r = 0.9706	5.44 ppm	69.10%
	150	0.4369		5.20 ppm	70.46%
Lower Methanol Isolate	50	0.4074	y = 0.048x + 0.7299	4.62 ppm	73.76%
	75	0.2973		2.45 ppm	86.06%
	100	0.2635		1.79 ppm	89.84%
	125	0.2344	r = 0.8443	1.22 ppm	93.09%
	150	0.2239		1.01 ppm	94.27%

Negative control absorbance= 1.0674

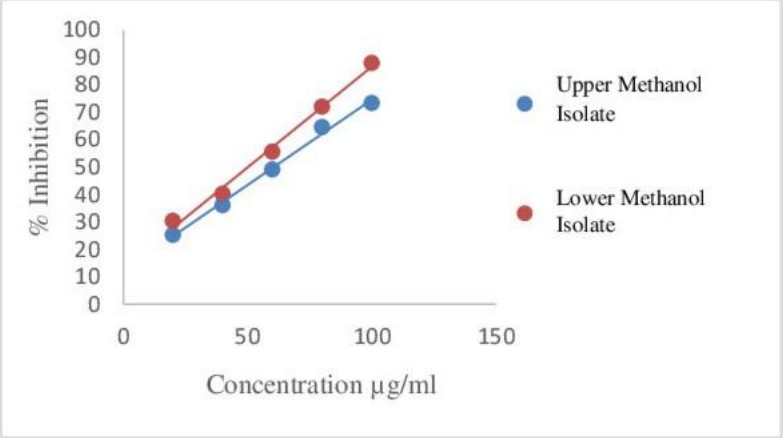

Figure 7. Anticholesterol activity curve of Upper and Lower Methanol Isolate

Discussion

An antioxidant activity test was carried out using bioassay-guided isolation through a phytochemical approach, starting with isolating natural compounds using certain methods. Antioxidant activity with an IC_{50} value indicates that the sample group and the positive control group had different activities as presented in Table 3 and Table 4. If the IC_{50} value is the same or close to the positive control, it can be said that the sample has the potential as one of the strongest antioxidant alternatives[26].

The methanol fraction has higher activity than the extract because the fraction contains more than one active compound based on differences in polarity so that these compounds synergize with each other to provide antioxidant activity, while the extract still has various groups of mixed secondary metabolite compounds so that the antioxidant activity is more fractional. The methanol fraction has a high activity effect as an antioxidant compared to the n-hexane fraction because methanol is a polar solvent where phenol is polar so that methanol is able to dissolve phenol better than other non-polar solvents[1]. Compounds with polar properties such as flavonoids have better activity in solubility properties with solvents. Meanwhile, the n-hexane fraction was less active but has potential as an antioxidant. This is because the sample contains few non-polar compounds[23]. Therefore, for the next process, the isolation was carried out on the methanol fraction as the most active fraction as an antioxidant.

The antioxidant activity of methanol isolates in Table 4 shows the difference in IC_{50} values of upper and lower methanol isolates related to the Rf values in the bands of the two isolates. The smaller the Rf value, the compound from the spot has a higher polarity than the larger Rf value, because the compound is more bound to the stationary phase is polar than the mobile phase[8]. Methanol fraction had higher antioxidant activity than methanol extract, n-hexane fraction, upper methanol isolate and lower methanol isolate. This is presumably because flavonoid compounds contributed greater antioxidant activity compared to extracts with many mixtures of other compounds and the isolates were single so that they only had 1 compound with the number of compounds contributing much less activity. Therefore, the fraction was more synergistic in antioxidant activity compared to extracts and isolates.

In reducing cholesterol, methanol extract at a concentration of 150 ppm was able to reduce cholesterol by 59.80%. This proves that the methanol extract of Parijoto fruit has anti-cholesterol activity in the presence of secondary metabolites such as flavonoids, tannins, and saponins. This result is greater than a study by Amin (2015) stating that the methanol extract of Parijoto with a concentration of 150 ppm can reduce cholesterol by 30.40%. The decrease in cholesterol in the methanol fraction of parijoto fruit with a concentration of 150 ppm was 43.86%. Naim (2017) mentioned that plant extracts contain active compounds of flavonoids, saponins, tannins, and terpenoids to lower cholesterol levels. Thus, the methanol extract of Parijoto has greater activity than the methanol fraction because it contains flavonoid compounds, saponins, and tannins.

The decrease in cholesterol levels in the n-hexane fraction with a concentration of 150 ppm was not significantly different from the methanol fraction, however descriptively, the decrease in cholesterol levels was smaller than the methanol fraction of 42.59%. Therefore, the isolation process was continued on the methanol fraction of parijoto fruit[3, 2, 15]. Lower methanol isolate with a concentration of 150 ppm was able to reduce cholesterol levels by 94.27% showing a significant difference compared to upper methanol isolates, where the same concentration was only able to reduce cholesterol levels by 70.47%. The decrease in cholesterol levels in lower methanol showing its high anticholesterol activity. The high anticholesterol activity in lower methanol

isolates was thought to be due to the presence of flavonoid secondary metabolites. This is in line with a study by Anggraini & Nabillah (2018) stating that plants containing flavonoids can lower cholesterol levels.

Conclusion

1) Based on the antioxidant activity test on extracts, fractions, and isolates of parijoto fruit, the methanol fraction had the highest antioxidant activity. The % inhibition level of extract, fraction, and isolate of parijoto fruit (Medinilla speciosa Blume) was 61.9927%, 64.3778%, 19.7652%, 49.7321%, and 57.2591%, Meanwhile, the IC_{50} value was 43.58 µg/ml, 40.64 µg/ml, 329.44 µg/m, 60.42 µg/ml, and 50.12 µg/ml.
2) Based on the anticholesterol activity test on extracts, fractions, and isolates of parijoto fruit, the lower methanol isolate had the highest cholesterol decrease. The decrease in cholesterol levels of the positive control, methanol extract, methanol fraction, n-hexane fraction, upper methanol isolate, and lower methanol isolate of parijoto fruit at concentrations of 50, 75, 100, 125, and 150 ppm, respectively, was methanol extract (50.74%, 53.48%, 54.79%, 56.31%, 59.80%), methanol fraction (28.98%, 31.02%, 41.92%, 42.49 %, 43.86%), n-hexane fraction (31.77%, 35.02%, 35.65%, 38.95%, 42.59%), upper isolate methanol (50.93%, 57, 20%, 62.15%, 69.10%, 70.46%), and lower lower methanol isolates (73.76%, 86.06%, 89.84%, 93.09%, 94.27%).

REFERENCES:

1. Adawiah, Sukandar, D., & Muawanah, A. The Activity of Antioxidant and Bioactive Component from Namnam Extract. Journal of Valence Chemistry. 2015; 1(2): 130–136.
2. Amin, M. S. Studi In-vitro : Efek Antikolesterol Dari Ekstrak Metanol Buah Parijoto (*Medinilla speciosa* Blume) Terhadap Kolesterol Total. Skripsi. 2015.
3. Anggraeni, Y. D., Wijayanti, R., & Utami, K. D. Pengaruh Pemberian Ekstrak Metanol, Fraksi Larut Dan Tak Larut N-Heksan Buah Parijoto (*Medinilla speciosa* Blume) Terhadap Diameter Tubulus Seminiferus Dan Jumlah Spermatosit Primer Tikus Jantan Galur Sprague dawley. Unissula. 2020.
4. Anggraini, D. I., & Nabillah, L. F. Activity Test of Suji Leaf Extract (*Dracaena angustifolia* Roxb.) on in vitro cholesterol lowering. Jurnal Kimia Sains Dan Aplikasi. 2018; 21(2): 54–58. https://doi.org/10.14710/jksa.21.2.54-58
5. Ayu, S. A., Pratiwi, L., & Nurbaeti, S. N. Uji kualitatif senyawa fenol dan flavonoid dalam ekstrak n-heksan daun senggani. Farmasi Fakultas Kedokteran Untan Pontianak. 2020; 1–6.
6. Djadjanegara, I., & Wahyudi, Pp. Uji Sitotoksisitas Ekstrak Etanol Herba Ceplukan (*Physalis angulata* Linn.) terhadap Sel T47D secara In Vitro. *Jurnal Ilmu Kefarmasian Indonesia*, *8*(1). 2010; 41–47. http://jifi.farmasi.univpancasila.ac.id/index.php/jifi/article/view/365
7. Esanda, H. Isolasi, Idenntifikasi dan Uji Aktivitas Antioksidan Senyawa Flavonoid Fraksi Klororform dan Fraksi Etil Asetat Daun Tanaman Adam Hawa (*Rhoe discolor* (L'her.) Hance) dengan Metode 2,2-diphenyl-1-picrylhidrazyl (DPPH). Skripsi USD Yogyakarta. 2016; 1–40.
8. Fatati, A. Potensi Antimalaria Kombinasi Ekstraksi Etanol Daun Widuri (*Calotropis gigantea*) dan Artemisin pada Mencit Terinfeksi Plasmodium berghei serta Identifikasi Senyawa Aktifnya. Skripsi UIN Maulana Malik Ibrahim Malang. 2014; 1–158.
9. Ferdinan, A., & Prasetya, A. B. Uji Aktivitas Antioksidan dari Ekstrak Jantung Pisang Kepok (*Musa paradisiaca* L.) Pontianak. Jurnal Ilmiah Ibnu Sina. 2018; 3(1): 88– 96.
10. Hasbullah, U. H., Pertiwi, R. B., Hidayah, I. N., & Andriyanti, D. Aktivitas Antioksidan Ekstrak Buah Parijoto Pada Berbagai Ph Pengolahan Pangan. Jurnal Ilmu-Ilmu Pertanian. 2020; *4*(2).
11. Isnindar, Wahyuno, S., & Setyowati, E. P. Isolasi Dan Identifikasi Senyawa Antioksidan Daun Kesemek (*Diospyros kaki* Thunb .) Dengan Metodedpph (2 , 2-Difenil-1- Pikrilhidrazil). Majalah Obat Tradisional. 2011; 16(3): 161–169. https://jurnal.ugm.ac.id/TradMedJ/article/view/8054/6245
12. Juliana, V. A., Aisyah, S., & Mustapha, I. Isolasi Dan Karakterisasi Senyawa Turunan Terpenoid Dari Fraksi N-Heksan *Momordica charantina* L. Jurnal Sains Dan Teknologi Kimia. 2010; 1(1): 88–93.
13. Kosman, R., & Tappang, K. Isolasi Dan Identifikasi Golongan Senyawa Kimia Fraksi Dietil Eter Daun Beruwas Laut (*Scaevola taccada* (Gaertn.) Roxb.) Asal Kabupaten Pinrang (Sulawesi Selatan). As-Syifaa. 2012; 04(02): 219–227.
14. Legawati, H. E., Kunarto, B., & Sani, Y. Fraksinasi Ekstrak Buah Parijoto (*Medinella Speciosa* L.) Dan Stabilitas Antosianinnya Pada Berbagai Lama Pemanasan. Jurnal Teknologi Hasil Pertanian Univrsitas Semarang. 2020. 15. Naim, F., Marianti, A., & Susanti, R. Aktivitas Ekstrak Daun Jati Belanda Terhadap Kadar Kolesterol HDL dan LDL pada Tikus Hiperkolesterolemia. Life Science. 2017; 6(1): 1–8.
16. Ningsih, W., Firmansyah, F., & Fitri, H. Formulasi Masker Peel Off dengan Beberapa Konsentrasi Ekstrak Etanol Buah Naga Super Merah (Hylocereus costaricensis (F.A.C Weber) Britton & Rose). Scientia : Jurnal Farmasi Dan Kesehatan. 2016; 6(1): 18. https://doi.org/10.36434/scientia.v6i1.37
17. Nishanthini, a, Ruba, a A., & Mohan, V. R. Total Phenolic , Flavonoid Contents And In Vitro Antioxidant Activity Of Leaf Of Suaeda Monoica Forssk Ex . Gmel (C*henopodiaceae*) International Journal Of Advanced Life Sciences (IJALS). International Journal of Advanced Life Sciences. 2012; 5(1): 34–43.
18. Niswah, L. Uji Aktivitas Antibakteri Dari Ekstrak Buah Parijoto (*Medinilla speciosa* Blume) Menggunakan Metode Difusi Cakram. Skripsi Program Studi Farmasi Fakultas Kedokteran Dan Ilmu Kesehatan Universitas Islam Negeri Syarif Hidayatullah Jakarta. 2014 September; 12–54.
19. Onkar, P., Bangar, J., & Karodi, R. Evaluation Of Antioxidant Activity Of Traditional Formulation Giloy Satva And Hydroalcoholic Extract Of The Curculigo Orchioides Gaertn. Journal of Applied Pharmaceutical Science. 2012; 2(6): 209–213. https://doi.org/10.7324/JAPS.2012.2733
20. Rezaeizadeh, A., Zuki, A. B. Z., Abdollahi, M., Goh, Y. M., Noordin, M. M., Hamid, M., & Azmi, T. I. Determination of antioxidant activity in methanolic and chloroformic extracts of *Momordica charantia*. African Journal of Biotechnology. 2011;

10(24): 4932–4940. https://doi.org/10.4314/ajb.v10i24.
21. Rosahdi, T. D., Kusmiyati, M., & Wijayanti, F. R. Uji Aktivitas Daya Antioksidan Buah Rambutan Rapiah Dengan Metode DPPH. Journal of Ocular Pharmacology and Therapeutics. 2013; 7(1): 362–363. https://doi.org/10.1089/jop.2007.0126
22. Setyowati, W. T., & Nisa, F. C. Formulasi Biskuit Tinggi Serat (Kajian Proporsi Bekatul Jagung : Tepung Terigu dan Penambahan Baking Powder). Jurnal Pangan Dan Agroindustri. 2014; 2(3): 224–231.
23. Wachidah, L. N. Uji Aktivitas Antioksidan Serta Penentuan Kandungan Fenolat Dan Flavonoid Total Dari Buah Parijoto (*Medinilla speciosa* Blume). Skripsi UIN Syarif Hidayatullah Jakarta. 2013; 1–69.
24. Wati, M., Erwin, & Tarigan, D. Isolasi dan Identifikasi Senyawa MetabolitSekunder dari Fraksi Etil Asetat pada Daun Berwarna Merah Pucuk Merah (Syzygium myrtifilium Walp.). Kimia FMIPA Unmul. 2017; 14(2): 100–107.
25. Wibowo, H., Wasino, & Setyowati, D. L. Kearifan Lokal Dalam Menjaga Lingkungan Hidup (Studi Kasus Masyarakat Di Desa Colo Kecamatan Dawe Kabupaten Kudus). Journal of Educational Social Studies. 2012; 1(1).
26. Widyowati, H., Ulfah, M., & Sumantri. Uji Aktivitas Antioksidan Ekstrak Etanolik Herba Alfalfa (*Medicago sativa* L.) dengan Metode DPPH (1,1-Diphenyl-2 Picrylhidrazyl). Jurnal Ilmu Farmasi Dan Farmasi Klinik. 2014; 11(1): 25–33.
27. Wijayanti, R., & Lestari, A. P. Pengaruh Ekstrak Etanolik Buah Parijoto (Medinilla Speciosa Blume) Terhadap Kadar Gula Darah Dan Fungsi Seksual Tikus Jantan Galur Wistar Model Diabetes Mellitus Kronik. *JIFFK :* Jurnal Ilmu Farmasi Dan Farmasi Klinik. 2018; 15(2): 1–7.