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In-vitro EVALUATION OF THE A-GLUCOSIDASE INHIBITORY, ANTI-CHOLESTEROL, AND DPPH SCAVENGING POTENTIAL OF LEAVE EXTRACTS OF *Rhaphidophora pinnata*

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ABSTRACT

Rhaphidophora pinnata (Lf) Schott leaves include alkaloids, saponins, flavonoids, tannins, steroids/triterpenoids, and glycosides, all of which are secondary metabolites with antioxidant, cholesterol-lowering, and anti-diabetic properties. Using n-hexane, ethyl acetate, and ethanol as solvents, layered percolation was used to extract. Anti-cholesterol activity using the Lieberman-Burchard Test and the antioxidant DPPH method (1,1-diphenyl-2-picryil hidrazin). The absorbance of antioxidant, antidiabetic, and anti-cholesterol activity was measured at a maximum wavelength of 516, 400, and 626 nm. The results of measurements of antioxidant activity, extracts of n-hexane, ethyl acetate, and ethanol of *Rhaphidophora pinnata* leaves obtained values of 516, 400, and 626 nm. The results of measurements of antioxidant activity, extracts of n-hexane, ethyl acetate, and ethanol of *Rhaphidophora pinnata* leaves obtained IC_{50} values of 74.2413; 27.8658; and 396,9975 µg/ml. The anti-cholesterol activity of the n-hexane extract with a concentration of 300 µg/ml was able to reduce cholesterol by 29.3395 % and ethyl acetate extract with a concentration of 35 µg/ml was 65.2030 %. Meanwhile, the ethanol extract of 100 µg/ml was 25.1489 %. Comparisons of fenofibrate and simvastatin with a concentration of 22.5 µg/ml of 65.8890 % and 76.3781 %. The results of the measurement of the antidiabetic activity of the extracts of n-hexane, ethyl acetate, and ethanol from the leaves of *Rhaphidophora pinnata*, aromas, and quercetin with IC_{50} values of 546.1511; 26.6306; 50.1951; 9.6862 and 2.4850 µg/ml. Ethyl acetate extract of *Rhaphidophora pinnata* leaves has an anti-cholesterol activity that is almost the same as fenofibrate at a concentration of 35 µg/ml.

Keywords: Antioxidant, Antidiabetic, Anti Cholesterol, *Rhaphidophora pinnata* (Lf) Schott, Lieberman-Burchard, UV-Vis Spectrophotometry

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INTRODUCTION

Lipid is one of the most caloric-dense energy sources. Apart from being a source of energy, lipids, particularly cholesterol, are a material that our bodies require and play a critical function in human life.[1] Based on the intake data from the individual dietary intake surveys, the mean cholesterol intake ranges from about 250-325 mg/body weight for men per day and 180-205 mg/ body weight for women per day.[2] Diabetes Mellitus is a chronic metabolic disorder defined by a constellation of symptoms brought on by abnormally high blood glucose levels (hyperglycemia). This is due to a pancreatic problem that results in elevated blood sugar levels. Diabetes mellitus is a significant hazard to human health and has climbed to become the seventh biggest cause of death worldwide.[3] Diabetes mellitus is a health disorder in the form of a group of symptoms that develop in a person as a result of an increase in blood sugar levels triggered by insulin deficiency or insulin resistance, as well as metabolic disorders in general.[4] If diabetes is not properly treated, it can lead to a variety of complications, both acute and chronic. Diabetes is a degenerative disease that cannot be cured but can be controlled.[5] According to the World Health Organization (WHO), 60% of total deaths in the world were caused by Non-Communicable Diseases (NCD) in 2010, which included

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DM.[6] One of the medicinal plants that are useful as a treatment for health is *Rhaphidophora pinnata* leaves. *Rhaphidophora pinnata* leaves contain secondary metabolites in the form of alkaloids, saponins, flavonoids, tannins, steroids/triterpenoids, and glycosides that shows high phenolic content.[7] The content of active compounds in *Rhaphidophora pinnata* leaves that are believed to reduce blood glucose levels and blood cholesterol levels are phenolic content. Several studies has indicated that phenolic content that has high antioxidant activity shows anti-diabetes and anticholesterol activity.[8-12] Therefore, it is necessary to determine a study regarding the activity of *Rhaphidophora pinnata* leaves as anti-diabetic and anti-cholesterol.

EXPERIMENTAL

Material and Instruments

The instruments used in this study were laboratory glassware, stirring rods, paper, mortar, microtube, analytical balance (Mettler Toledo), and micropipette (Eppendorf; 100-1000 µL), UV-Vis spectrophotometer (Shimadzu). The materials used in this study were extracts of n-hexane, ethyl acetate, and ethanol from *Rhaphidophora pinnata* leaves which were extracted by percolation and pro-analysis and technical quality materials, Dimethyl sulfoxide (DMSO), phosphate buffer pH 7, enzyme solution, p-nitrophenyl-α-D-glucopyranoside (PNPG), and sodium carbonate.

Antioxidant Activity Examination

An antioxidant activity test was conducted using the DPPH method with a concentration of 40 µg/ml on n-hexane, ethyl acetate, and ethanol extract of *Rhaphidophora pinnata* leaves with various concentrations added by 1 ml of DPPH 200 µg/ml standard solvent. Then, the volume was sufficient with methanol to the mark and homogenized and let stand for 15 minutes. After that, the absorption was measured using a UV-Visible spectrophotometer.[13]

In vitro Anticholesterol Examination

The extract was tested in the form of a solution in chloroform, adding to 2.5 ml of standard cholesterol solution (concentration of 400 µg/ml). After being homogenized, 2 ml of anhydrous acetic acid and 0.1 ml of concentrated sulfuric acid were added. Then, chloroform was added and the solution was left in the dark place for 15 minutes. At last, the absorption was measured using a UV-Visible spectrophotometer with a cholesterol solution with a concentration of 400 µg/ml as a negative control.[14]

α-glucosidase Inhibitory Activity

Reacted 5 µL of sample solution of various concentrations added with 245 µL of phosphate buffer pH 7 and 125 µL of phosphate buffer pH 7, incubated for 5 minutes at room temperature. 125 µL p-nitrophenyl-α-D-glucopyranoside (PNPG) was added at a concentration of 5 mM. Samples were incubated for 15 minutes at room temperature. 1000 µL of 200 mM sodium carbonate was added. The absorbance of the sample was measured using a spectrophotometer at the maximum wavelength obtained.[15]

RESULTS AND DISCUSSION

Results of Antioxidant Activity Test

The maximum absorbance wavelength at the DPPH standard produced a maximum absorption wavelength of 516 nm. DPPH absorption spectrum can be seen in Fig.-1.

The IC_{50} value for each of the extracts with n-hexane, ethyl acetate, and ethanol solvents was determined using a linear regression equation from the curve of the sample concentration to percent inhibition with the equation Y = ax + b, sample concentration (ppm) as the (X) axis. and the value of the proportion of inhibition as the (Y) axis. Regression equation for n-hexane extract was y = 0.1135X + 4.9249, ethyl acetate was y = 1.5893X + 5.7142 and ethanol extract was y = 0.7304X - 4.2234. The results of antioxidant activity of n-hexane, ethyl acetate, and ethanol extract of *Rhaphidophora pinnata* leaves can be seen in Tables-1 to 3.

Table-1: Results of Antioxidant Activity of n-Hexane Extract

Sample	Concentration (ppm)	absorbance	% inhibition	IC_{50} (µg/ml)
n-hexane extract	0	0.9661	0	396.9975
	100	0.7977	19.9177	
	200	0.6891	30.8202	

	300	0.5450	39.6145	
	400	0.5021	49.5934	
	500	0.3993	59.9137	

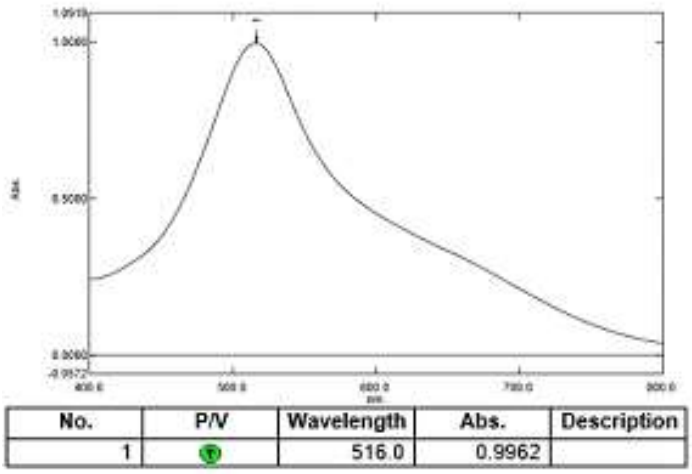


Fig.-1: The Maximum Absorbance Wavelength of DPPH

Table-2: Results of Antioxidant Activity of Ethyl Acetate Extract

Sample	Concentration (ppm)	absorbance	% inhibition	IC_{50} (µg/ml)
ethyl acetate extract	0	0.9661	0	27.8658
	10	0.7793	21.7649	
	20	0.5450	45.2866	
	30	0.5450	56.4000	
	40	0.3203	67.8446	
	50	0.1855	81.3774	

Table-3: Results of Antioxidant Activity of Ethanol Extract

Sample	Concentration (ppm)	absorbance	% inhibition	**IC_{50} (µg/ml)**
Ethanol extract	0	0.9661	0	74.2413
	50	0.7396	25.7504	
	60	0.6396	35.7896	
	70	0.5450	45.9191	
	80	0.4406	55.7675	
	90	0.3281	67.0615	

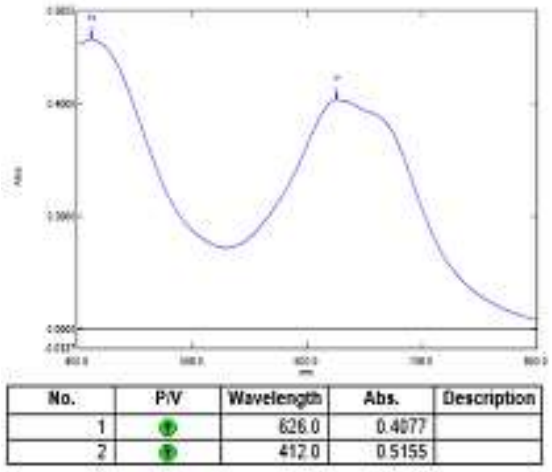
The value of IC_{50} is inversely proportional to the antioxidant activity, the higher the antioxidant activity, the lower the IC_{50} value. Table-2 presents that the ethyl acetate extract of *Rhaphidophora pinnata* leaves had the highest antioxidant activity compared to n-hexane and ethanol extracts with the IC_{50} value of 27.8658 µg/ml.[16] This is presumably since the *Rhaphidophora pinnata* leaf samples contain many bioactive compounds that are semipolar compared to nonpolar and polar bioactive compounds. Therefore, the semipolar solvent in this study, ethyl acetate, attracted more bioactive components in the *Rhaphidophora pinnata* leaves.

Results of Anti-Cholesterol Activity

The maximum absorbance wavelength at the cholesterol standard resulted in a maximum absorption wavelength of 626 nm. The cholesterol absorption spectrum is visualized in the following Fig.-2. The absorption spectrum of cholesterol at various concentrations can be seen in Fig.-3.

The cholesterol absorption curve showed that the value of r = 0.9995 was obtained with the regression equation of Y = 0.0067x + 0.0018. Good linearity results were obtained if the regression coefficient is

between 0.8-1.[16] The results of invitro anticholesterol activity from the extract of n-hexane, ethyl acetate, ethanol of *Rhaphidophora pinnata* leaves, fenofibrate, and simvastatin are presented in Table-4.



No.	P/V	Wavelength	Abs.	Description
1		626.0	0.4077	
2		412.0	0.5155	

Fig.-2: The Maximum Absorbance Wavelength of Cholesterol

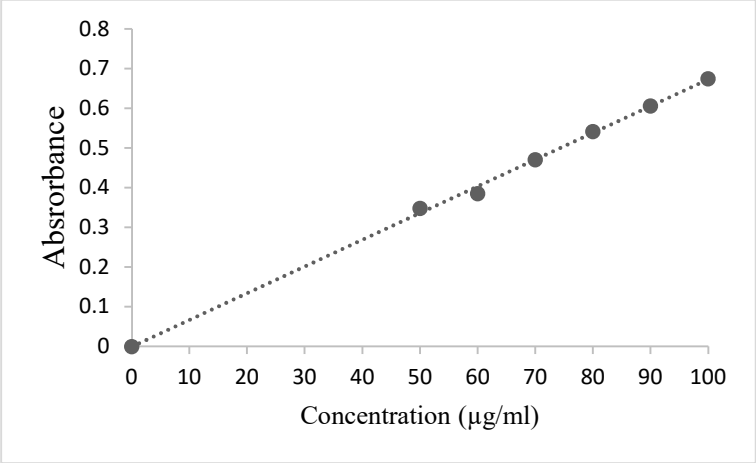


Fig.-3: Cholesterol Calibration Curve

Table-4: Result of Invitro Anti-Cholesterol Activity

Sample	Dose Concentration (µg/ml)	Cholesterol Level (µg/ml)	% Reduction
Negative Control	-	99.3681	0
n-hexane extract	100	89.1940	10.2390
	200	81.5045	17.9771
	300	70.2140	29.3395
	400	63.0646	36.5343
	500	54.3482	45.4463
Ethyl acetate extract	15	85.1990	14.2592
	20	72.4895	27.0495
	25	59.9293	39.6896
	30	46.2090	53.4971
	35	34.5772	65.2030
Ethanol extract	25	92.2040	7.2096
	50	86.3133	13.1378
	75	79.8457	19.6465
	100	74.3781	25.1489
	125	66.5373	33.0395

Fenofibrate	12.5	40.7711	58.9696
	15	38.4029	61.3529
	17.5	37.2288	62.5344
	20	35.6019	64.1717
	22.5	33.8954	65.8890
Simvastatin	12.5	38.6218	61.1326
	15	34.7512	65.0278
	17.5	30.5770	69.2285
	20	26.4975	73.3339
	22.5	23.4726	76.3781

Table-3 shows that the ethanol extract of *Rhaphidophora pinnata* leaves with a concentration of 25 µg/ml can reduce cholesterol by 7.2096 %. Ethyl acetate extract with a concentration of 15 µg/ml was able to reduce cholesterol by 14.2592 %. N-hexane extract with a concentration of 100 µg/ml was able to reduce cholesterol by 10.2390 %. This demonstrates that *Rhaphidophora pinnata* leaf extracts in ethanol, ethyl acetate, and n-hexane have been shown to have cholesterol-lowering action. At a concentration of 15 g/ml, ethyl acetate extract of *Rhaphidophora pinnata* leaves demonstrated stronger effectiveness in lowering cholesterol levels than ethanol and n-hexane extracts. It can lower cholesterol levels by 14.2592 %, whereas ethanol and n-hexane extracts at concentrations of 25 g/ml and 100 g/ml can only lower cholesterol levels by 7.2096 % and 10.2390 %, respectively. The activity of reducing cholesterol levels of n-hexane, ethyl acetate, and ethanol from *Rhaphidophora pinnata* leaves compared to fenofibrate and simvastatin had lower cholesterol-lowering activity. The comparison of fenofibrate and simvastatin with a concentration of 12.5 µg/ml was able to reduce cholesterol by 58.9696 % and 61.1326 %. The absorbance value shown by each concentration was different. The higher the sample concentration, the lower the absorbance value. This occurred because the high sample concentration is capable of significantly lowering cholesterol levels. As a result, the absorbance value was reduced, but the percentage of anti-cholesterol action was increased. The maximum activity was observed in the ethyl acetate extract, since ethyl acetate was attracted to a greater number of secondary metabolites, such as flavonoids. Phenolics, flavonoids, and vitamin C all appear to play a role in decreasing cholesterol levels. The hydroxyl group on cholesterol reacts with the ketone group on the flavonoids to form a hemiacetal.[17] The carbonyl groups on the flavonoids might react with the hydroxyl groups on cholesterol to form hydrogen bonds.[18] The compound that is not bound by this sample is called free cholesterol which reacts with anhydrous acetic acid and concentrated sulfuric acid.

Results of Antidiabetic Activity Test

The standard maximum absorbance wavelength of α-glucosidase yields a maximum absorption wavelength of 400 nm. The maximum absorption spectrum can be seen in Figure-4.

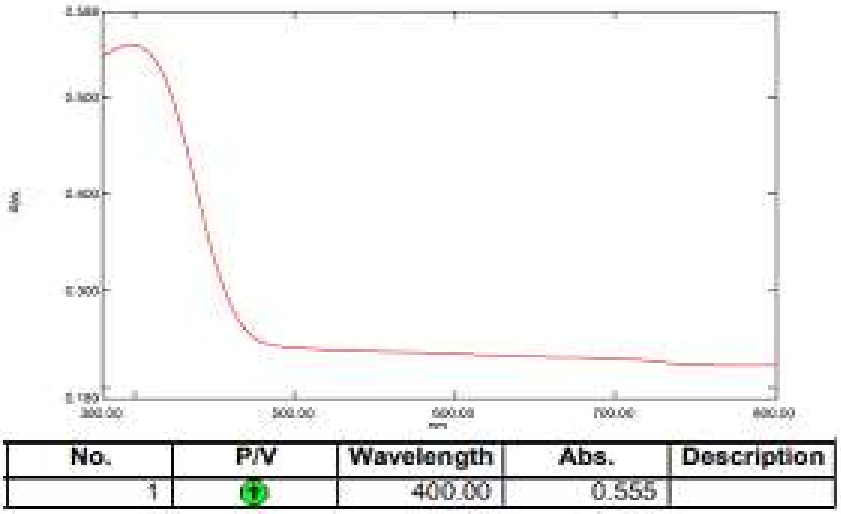


Fig.-4: Maximum Absorption Wavelength of α-glucosidase

The results of the antidiabetic activity of the extracts of n-hexane, ethyl acetate, ethanol of *Rhaphidophora pinnata* leaves, acarbose, and quercetin are presented in Table-5 to 9.

Tables- 5 to 9 present that the extracts of n-hexane, ethyl acetate, and ethanol have antidiabetic activity with IC_{50} values of 546.1511, 26.6306, and 50.1951, respectively. Ethyl acetate extract had greater antidiabetic activity compared to n-hexane and ethanol extracts. Compared to acarbose and quercetin, the extracts of n-hexane, ethyl acetate, and ethanol had lower antidiabetic activity seen from the IC_{50} value. The higher the concentration of the material, the higher the absorbance value, and hence the larger the percentage of antidiabetic activity. The antidiabetic activity test was conducted on acarbose and quercetin since acarbose is a regularly used antidiabetic in Indonesia. Additionally, it is readily available and frequently used as a comparison in a variety of publications. The percentage of the inhibition value was calculated by comparing the absorbance of the sample (S) and the absorbance of blank (B). Furthermore, the percentage of inhibition is used to calculate the IC_{50} value. The results indicated that *Rhaphidophora pinnata* shows inhibition activity on α-glucosidase by in-vitro test and it showed a dose-dependent manner.

Table-5: Results of the Antidiabetic Activity Test of Ethanol Extract of *Rhaphidophora pinnata*

Concentration (µg/ml)		Absorbance			Mean of Absorbance	S_1-S_0	% Inhibition	IC_{50}
		1	2	3				
Blank (No Extract)	S_1	0.9171	0.9167	0.9154	0.9164	0.8785	0	50.1951
	S_0	0.0384	0.0376	0.0377	0.0379			
20 µg/ml	S_1	0.5772	0.5771	0.5773	0.5772	0.5498	37.416	
	S_0	0.0274	0.0274	0.0275	0.0274			
40 µg/ml	S_1	0.4758	0.4755	0.4751	0.4755	0.4503	48.7421	
	S_0	0.0251	0.0252	0.0253	0.0252			
60 µg/ml	S_1	0.3754	0.3754	0.3753	0.3754	0.3524	59.8862	
	S_0	0.0230	0.0231	0.0230	0.0230			
80 µg/ml	S_1	0.2862	0.2859	0.2861	0.2861	0.2621	70.165	
	S_0	0.0241	0.0241	0.0239	0.0240			
100 µg/ml	S_1	0.1799	0.1801	0.1801	0.1800	0.1510	82.914	
	S_0	0.0289	0.0291	0.0289	0.0290			

Table-6: Results of the Antidiabetic Activity Test of Ethyl Acetate Extract of *Rhaphidophora pinnata*

Concentration (µg/ml)		Absorbance			Mean of Absorbance	S_1-S_0	% Inhibition	IC_{50}
		1	2	3				
Blank (No Extract)	S_1	0.9171	0.9167	0.9154	0.9164	0.8785	0	26.6306
	S_0	0.0384	0.0376	0.0377	0.0379			
10 µg/ml	S_1	0.5643	0.5642	0.5641	0.5642	0.5343	39.1804	
	S_0	0.0300	0.0299	0.0299	0.0299			
20 µg/ml	S_1	0.4850	0.4853	0.4850	0.4851	0.4470	49.1178	
	S_0	0.0381	0.0381	0.0381	0.0381			
30 µg/ml	S_1	0.4130	0.4125	0.4125	0.4127	0.3738	57.4502	
	S_0	0.0388	0.0390	0.0390	0.0389			
40 µg/ml	S_1	0.3261	0.3262	0.3262	0.3262	0.2864	67.3989	
	S_0	0.0399	0.0398	0.0397	0.0398			
50 µg/ml	S_1	0.2682	0.2684	0.2684	0.2683	0.2291	73.9214	
	S_0	0.0392	0.0391	0.0392	0.0392			

Table-7: Results of the Antidiabetic Activity Test of n-Hexane Extract of *Rhaphidophora pinnata*

Concentration (µg/ml)		Absorbance			Mean of Absorbance	S_1-S_0	% Inhibition	IC_{50}
		1	2	3				
Blank (No Extract)	S_1	0.9171	0.9167	0.9154	0.9164	0.8785	0	546.1511
	S_0	0.0384	0.0376	0.0377	0.0379			
200 µg/ml	S_1	0.7431	0.7431	0.7431	0.7431	0.7081	19.3967	
	S_0	0.0350	0.0351	0.0349	0.0350			
350 µg/ml	S_1	0.6242	0.6243	0.6244	0.6243	0.5878	33.0904	

	S_0	0.0366	0.0365	0.0365	0.0365			
500 µg/ml	S_1	0.5110	0.5110	0.5109	0.5110	0.4726	46.2037	
	S_0	0.0384	0.0384	0.0384	0.0384			
650 µg/ml	S_1	0.4001	0.4003	0.4003	0.4002	0.3617	58.8275	
	S_0	0.0384	0.0385	0.0385	0.0385			
800 µg/ml	S_1	0.2798	0.2799	0.2799	0.2799	0.2409	72.5782	
	S_0	0.0390	0.0389	0.0391	0.0390			

Table-8: Results of the Antidiabetic Activity Test of Quercetin

Concentration (µg/ml)		Absorbance			Mean of Absorbance	S_1-S_0	% Inhibition	IC_{50}
		1	2	3				
Blank (No Extract)	S_1	0.9171	0.9167	0.9154	0.9164	0.8785	0	2.485
	S_0	0.0384	0.0376	0.0377	0.0379			
1 µg/ml	S_1	0.6828	0.6825	0.6826	0.6826	0.6510	25.8964	
	S_0	0.0319	0.0315	0.0314	0.0316			
2 µg/ml	S_1	0.5260	0.5261	0.5260	0.5260	0.5011	42.9595	
	S_0	0.0249	0.0249	0.0249	0.0249			
3 µg/ml	S_1	0.3709	0.3708	0.3708	0.3708	0.3490	61.07	
	S_0	0.0289	0.0288	0.0288	0.0288			
4 µg/ml	S_1	0.2520	0.2518	0.2520	0.2519	0.2246	74.4337	
	S_0	0.0274	0.0274	0.0272	0.0273			
5 µg/ml	S_1	0.0737	0.0737	0.0737	0.0737	0.0124	95.5885	
	S_0	0.0364	0.0362	0.0362	0.0364			

Table-9: Results of the Antidiabetic Activity Test of Acarbose

Concentration (µg/ml)		Absorbance			Mean of Absorbance	S_1-S_0	% Inhibition	IC_{50}
		1	2	3				
Blank (No Extract)	S_1	0.9171	0.9167	0.9154	0.9164	0.8785	0	9.6862
	S_0	0.0384	0.0376	0.0377	0.0379			
5 µg/ml	S_1	0.6870	0.6869	0.6870	0.6870	0.6672	24.0524	
	S_0	0.0198	0.0198	0.0198	0.0198			
7.5 µg/ml	S_1	0.6168	0.6168	0.6168	0.6168	0.5959	32.1684	
	S_0	0.0209	0.0209	0.0210	0.0209			
10 µg/ml	S_1	0.5342	0.5341	0.5343	0.5342	0.5084	42.1286	
	S_0	0.0258	0.0258	0.0259	0.0258			
12.5 µg/ml	S_1	0.4436	0.4435	0.4436	0.4436	0.4217	51.9977	
	S_0	0.0218	0.0217	0.0219	0.0219			
15 µg/ml	S_1	0.3037	0.3037	0.3035	0.3037	0.2839	67.6835	
	S_0	0.0198	0.0198	0.0198	0.2839			

CONCLUSION

The results of the study and observations indicate that the ethyl acetate extract of *Rhaphidophora pinnata* leaves has the highest antioxidant activity compared to the ethanol and n-hexane extracts with an IC_{50} value of 27.8658 µg/ml. Extracts of n-hexane, ethyl acetate, and ethanol from *Rhaphidophora pinnata* leaves have anti-cholesterol and antidiabetic activities with lower activity compared to fenofibrate, simvastatin, acarbose, and quercetin.

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