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*Original Research Article*

# Evaluation of Antihyperlipidemic and Antidiabetic Activity of *Phyllanthus emblica* L. Fruits

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ABSTRACT

*Phyllanthus emblica* L. fruit is used traditionally as medicine for diarrhea, thrush, and cholesterol and it has been studied that *Phyllanthus emblica* L. fruit have various secondary metabolites such as alkaloids, saponins, flavonoids, tannins, steroids/triterpenoids, glycosides and it also contains high vitamin C, which has anti-cholesterol and anti-diabetic effect. The purpose of the research was to evaluate the anti-cholesterol and anti-diabetic activity of ethanol extract of *P. emblica* fruit. The extract was obtained by percolation with 96% ethanol solvent. The anti-cholesterol activity was carried out using the *Lieberman-Burchard* method while the anti-diabetic activity was assessed using the alpha-glucosidase inhibitory activity assay. The determination of the anti-cholesterol activity of the ethanol extract of *P. emblica*, fenofibrate and simvastatin was carried out at serial concentrations (12.5; 15; 17.5; 20; and 22.5 µg/mL). Ethanol extract of *P. emblica* fruit reduce cholesterol level by 67.2608; 68.6076; 69.9194; 72.0773 and 73.4992% at 12.5; 15; 17.5; 20; and 22.5 µg/mL, respectively. Fenofibrate showed anti-cholesterol activity with percentage reduction of 58.9696; 61.3529; 62.5344; 64.1717 and 65.8890% while Simvastatin showed anti-cholesterol activity with percentage reduction of 61.1326; 65.0278; 69.2285; 73.3339 and 76.3781% at 12.5; 15; 17.5; 20; and 22.5 µg/mL, respectively. The $IC_{50}$ values for alpha-glucosidase inhibition for *P. emblica* extract, acarbose and quercetin were 4.5833; 9.6862 and 2.4850 µg/mL, respectively. It can be concluded that the ethanol extract of *P. emblica* fruit has anti-cholesterol activity that is similar to simvastatin and it showed higher activity than fenofibrate and it also showed higher antidiabetic activity than acarbose.

***Keywords*:** Anticholesterol, Antidiabetic, Ethanol extracts, *Phyllanthus emblica L*., Lieberman Burchard, Spectrophotometry

## Introduction

Diet really determines a person's health condition. A diet that contains more fiber from both vegetables and fruits will reduce the risk of suffering from degenerative diseases, cardiovascular disease, diabetes, and cancer. Otherwise, foods that contain less or no fiber, diseases as mentioned above will increase and will reduce the quality of life of individuals suffering from these diseases.[1] Nowadays food patterns have changed, people prefer to eat fast food which do not have a good balance of nutrients to meet nutritional needs of the body. Fast food contains more fat, cholesterol and little or no fiber. This nutritional imbalance will cause many problems for individuals who like to consume these foods.[1]

Cholesterol is a component of lipid. Lipid is one of the nutrients that is needed in the body in addition to other nutrients, such as carbohydrates, protein, vitamins and minerals. Lipid is one of the energy sources that provide the highest calories. Apart from being a source of energy, lipid substance particularly cholesterol is needed in our bodies and has an important role in human life.[2]

Cholesterol substance is very difficult to avoid from the human diet, to be able to avoid cholesterol intake requires significant dietary changes. This dietary changes are needed to avoid the risk of coronary artery disease (CHD).[3]

Diabetes mellitus (DM) is a disease with a set of symptoms that occurs through the increase of blood glucose levels (hyperglycemia) and is a chronic metabolic disease. This is due to a disturbance in the pancreas, resulting in excess sugar in the blood. The global prevalence of diabetes were estimated to be 9.3% (463 million people) in 2019, and rising to be 10.2% (578 million) in 2030 and 10.9% (700 million) in 2045.[4]

One of the medicinal plants that is beneficial for health is *Phyllanthus emblica* L. *P. emblica* fruit has several pharmacological benefits for example anti-inflammatory, antioxidant, antibacterial, antifungal, antidiabetic, anticancer, and antidepressant.[5-7] Fruit of *P. emblica* contain secondary metabolites namely flavonoids, alkaloids, and tannins.[8] The purpose of the research was to evaluate the anti-cholesterol and anti-diabetic activity of ethanol extract of *P. emblica* fruit by extracted *Phyllanthus emblica* L. using percolation method in 96% ethanol solvent. The determination of cholesterol reduction levels was carried out using a UV-Vis spectrophotometer with the method of Lieberman-Burchard [9], and the antidiabetic activity was carried out by *A*-glucosidase Enzyme Activity Inhibition Test.

## Materials and Methods

*Materials*

Fruits of *Phyllantus emblica* were taken from Simardona Village, District Batang Onang, Regency Padang Lawas Utara, Province North Sumatera in August 2018. Identification was carried out at Herbarium

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*Extraction of Phyllantus emblica fruit*
As many as 600 g of dried sample of *P. emblica fruit* was extracted with 21 L of 96% ethanol solvent by percolation method. After which the solvent was evaporated using rotary evaporator at a temperature of 40-50°C until a thick mass is obtained.

*Determination of antihyperlipidemic activity of ethanol extract of Phyllanthus emblica L. and positive control (fenofibrate and simvastatin)*
The ethanol extract of *P. emblica*, fenofibrate and simvastatin was weighed at 25 mg. The weighed samples were dissolved with Chloroform to get a total volume of 25 mL each with serial dilutions were prepared at varied concentrations (12.5; 15; 17.5; 20; and 22.5 µg/mL). The solutions were allowed to homogenize after which 2 mL of anhydrous acetic acid and 0.1 mL conc. sulfuric acid was added. The absorption test was measured using a UV-Visible spectrophotometer at 626 nm.[10]

*Alpha-glucosidase enzyme activity inhibition test*
5 µL of samples of various concentrations were reacted with 245 µL of phosphate buffer pH 7 and 125 µL of 0.15 U/mL enzyme solution, incubated for 5 minutes at room temperature. 125 µL of p-nitrophenyl-*α*-D-glucopyranoside (PNPG) at a concentration of 5 mM was added. The samples were incubated again for 15 minutes at room temperature. After the incubation was complete, 1000 µL of 200 mM sodium carbonate was added. The absorbance of the sample was read using a spectrophotometer at 400 nm.[11]

*Statistical analysis*
The data were analyzed using LSD (*Least Significance Difference)* test with p value ($p < 0.05$).

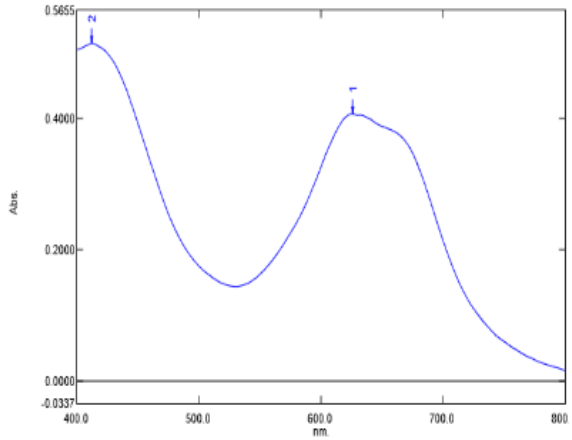
## Results and Discussion

The maximum absorbance results on the cholesterol standard resulted in the maximum absorption wavelength of 626 nm. The cholesterol absorption spectrum is shown in Figure 1.

*Decreased cholesterol levels*
The result of reducing cholesterol levels in the ethanol extract of the fruit *P. emblica,* fenofibrate and simvastatin can be seen in Table 1.
From Table 1 it is known that the ethanol extract of the fruit *P. emblica* with a concentration of 12.5 µg/mL was able to reduce cholesterol by 67.2608%. This indicates that the ethanol extract of the fruit *P. emblica* has proven to have the ability to reduce cholesterol levels *in vitro* because of the content of secondary metabolites such as tannins, saponins, and flavonoids.
The ethanol extract of *P. emblica* fruit at a concentration of 12.5; 15 and 17.5 µg/mL showed a greater activity in inhibiting cholesterol levels by in vitro test compared with a simvastatin but at the concentrations of 20 and 22.5 µg/mL, simvastatin showed a greater inhibition in lowering cholesterol levels.
The LSD test results in table 2 indicate that the ethanol extract of the fruit *P. emblica* showed a significant difference in the 95% confidence level when compared with fenofibrate.
Fruit ethanol extract *P. emblica* had a better effect but did not show a significant difference.

*Antidiabetic activity*
The standard maximum absorbance of *α*-glucosidase resulted in a maximum absorption wavelength of 400 nm. The maximum absorption spectrum can be seen in Figure 3.
Results of the antidiabetic activity of the ethanol extract of the fruit P. emblica, acarbose and quercetin can be seen in Table 3 - 6 and Figure 4-6. From Table 6 above it is known that the ethanol extract of *P. emblica* fruit, acarbose and quercetin have antidiabetic activity with $IC_{50}$ is 4,5833; 9,6862 and 2,4850.

*Phylanthus emblica* L. contains of alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids and glycosides. Other study showed that *P. emblica* fruit also contains another phenolic compounds, such as geraniin, quercetin3-*β*-D-glukopiranosida, kaempferol3-*β*-D-glukosapiranosida, isokorilagin, quercetin, and kaempferol[12]. Besides, this plant also contains gallic acid, ellagic acid, 1-O-galloyl-beta-D-glukosa, acid-3-ethylgalic acid and corilagin. Apigenin dan ascorbic acid have also been found in *P. emblica* plants. The hydroxyl groups which are acidic in phenolic compounds are thought to play a very important role in the oxidation-reduction reactions that occur in the body.[13] The compounds that are thought to play a role in lowering cholesterol levels are phenolic, flavonoid, and vitamin C.[14] The hydroxyl group on cholesterol reacts with the ketone groups on the flavonoids to form hemiacetals. The carbonyl groups on the flavonoids will react with the hydroxyl groups on cholesterol to form hydrogen bonds. The compound that is not bound by this sample or called free cholesterol reacted with anhydrous acetic acid and sulfuric acid and this reaction was measured using a spectrophotometer.[1] *P. emblica* extract showed a higher activity in the reduction of cholesterol levels compared to fenofibrate, but it showed a lower activity when compared to simvastatin. The inhibition of the *α*-glucosidase enzyme also can be analyzed from the $IC_{50}$ value. The $IC_{50}$ of *α*-glucosidase inhibition test of ethanol extract of *P. emblica fruit*, acarbose, and quercetin showed $IC_{50}$ value at a concentration of 4.5833, 9.6862 and 2.4850 µg/ml respectively. The ethanol extract of *P. emblica* fruit showed inhibiting activity of *α*-glucosidase enzyme and was stronger than acarbose which had $IC_{50}$ of 9.6862 µg/mL.




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

**Figure 1:** The maximum absorbance wavelength of cholesterol

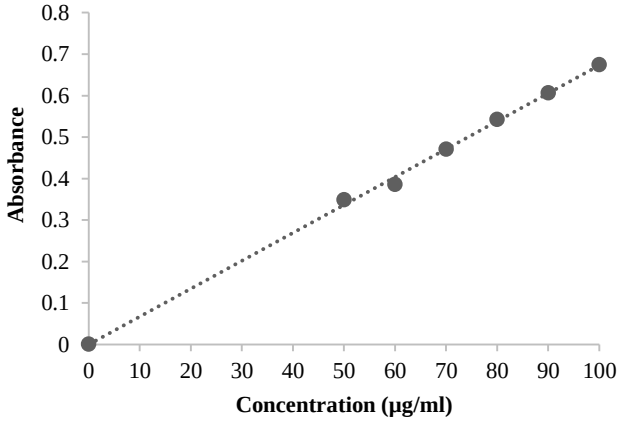



**Figure 2:** Cholesterol Calibration Curve

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**Table 1:** Lower cholesterol levels

| Sample | Concentration (µg/mL) | % Reduction of Cholesterol levels | | |
|---|---|---|---|---|
| | | E. *P. emblica* | Fenofibrate | Simvastatin |
| Negative Control | Cholesterol (100 µg/mL) | | 99.3681 | |
| | 12.5 | 67.2608 | 58.9696 | 61.1326 |
| | 15 | 68.6076 | 61.3529 | 65.0278 |
| | 17.5 | 69.9194 | 62.5344 | 69.2285 |
| | 20 | 72.0773 | 64.1717 | 73.3339 |
| | 22.5 | 73.4992 | 65.8890 | 76.3781 |

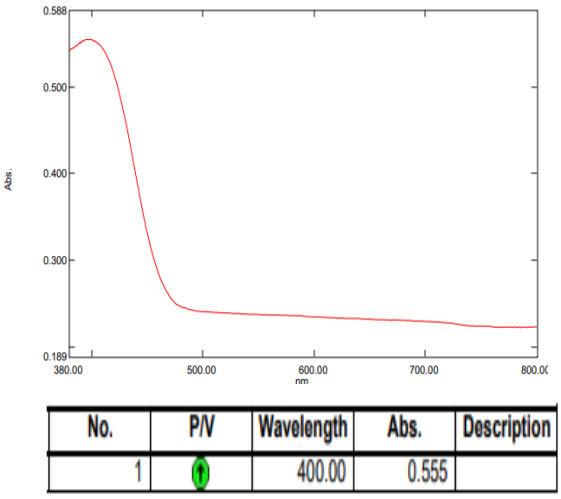


**Figure 3:** Maximum absorbance wavelength

**Table 2:** Test result of LSD

| Sample | Sample | Difference Average | Standard Error | Significance | Confidence Interval 95 % | |
|---|---|---|---|---|---|---|
| | | | | | Lower limit | Upper limit |
| E. *P. emblica* | Simvastatin | 1.252680 | 1.3125905 | 0.368 | -1.774159 | 4.279519 |
| | fenofibrate | 7.689340* | 1.3125905 | 0.000 | 4.662501 | 10.716179 |
| Simvastatin | E. *P. emblica* | -1.252680 | 1.3125905 | 0.368 | -4.279519 | 1.774159 |
| | Fenofibrate | 6.436660* | 1.3125905 | 0.001 | 3.409821 | 9.463499 |
| Fenofibrate | E. *P. emblica* | -7689340* | 1.3125905 | 0.000 | -10.716179 | -4.662501 |
| | Simvastatin | -6.436660* | 1.3125905 | 0.001 | -9.463499 | -3.409821 |

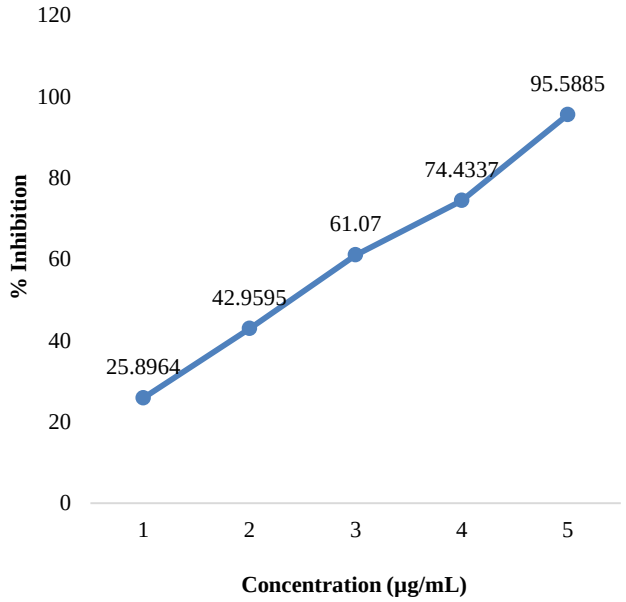


**Figure 4:** Graph % inhibition of *P.emblica* fruit ethanol extract

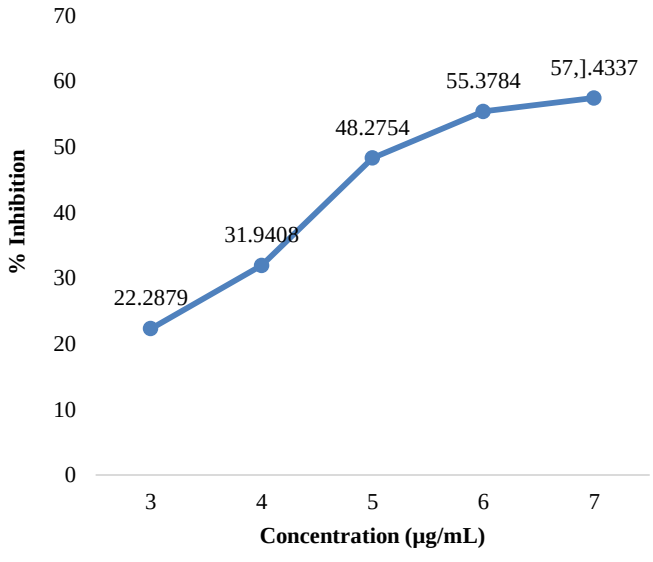


**Figure 5:** Graph % inhibition of acarbose

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**Table 3:** Antidiabetic Activity of *P. emblica* Fruit Ethanol Extract

| Concentration (µg / ml) | | Absorbance | | | Absorbance Average | $S_1$-$S_0$ | % Inhibition | $IC_{50}$ |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | | | |
| Blank (No Extract) | $S_1$ | 0.9171 | 0.9167 | 0.9154 | 0.9164 | | 0 | |
| | $S_0$ | 0.0384 | 0.0376 | 0.0377 | 0.0379 | 0.8785 | | |
| | $S_1$ | 0.7231 | 0.7229 | 0.7229 | 0.7230 | | | |
| 3 µg/mL | $S_0$ | 0.0404 | 0.0403 | 0.0404 | 0.0403 | 0.6827 | 22.2879 | |
| | $S_1$ | 0.6186 | 0.6184 | 0.6179 | 0.6183 | | | |
| 4 µg/mL | $S_0$ | 0.0204 | 0.0203 | 0.0205 | 0.0204 | 0.5979 | 31.9408 | |
| | $S_1$ | 0.5065 | 0.5065 | 0.5063 | 0.5064 | | | 4.5833 |
| 5 µg/mL | $S_0$ | 0.0521 | 0.0520 | 0.0519 | 0.0520 | 0.4544 | 48.2754 | |
| | $S_1$ | 0.4266 | 0.4266 | 0.4266 | 0.4266 | | | |
| 6 µg/mL | $S_0$ | 0.0345 | 0.0346 | 0.0346 | 0.0346 | 0.3920 | 55.3784 | |
| | $S_1$ | 0.3203 | 0.3202 | 0.3202 | 0.3202 | | | |
| 7 µg/mL | $S_0$ | 0.0296 | 0.0296 | 0.0296 | 0.0296 | 0.2906 | 57.4337 | |

**Table 4:** Acarbose antidiabetic activity

| Concentration (µg / mL) | | Absorbance | | | Absorbance Average | $S_1$-$S_0$ | % Inhibition | $IC_{50}$ |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | | | |
| Blank (No Extract) | $S_1$ | 0.9171 | 0.9167 | 0.9154 | 0.9164 | | | |
| | $S_0$ | 0.0384 | 0.0376 | 0.0377 | 0.0379 | 0.8785 | 0 | |
| | $S_1$ | 0.6870 | 0.6869 | 0.6870 | 0.6870 | | | |
| 5 µg/mL | $S_0$ | 0.0198 | 0.0198 | 0.0198 | 0.0198 | 0.6672 | 24.0524 | |
| | $S_1$ | 0.6168 | 0.6168 | 0.6168 | 0.6168 | | | |
| 7,5 µg/mL | $S_0$ | 0.0209 | 0.0209 | 0.0210 | 0.0209 | 0.5959 | 32.1684 | 9.6862 |
| | $S_1$ | 0.5342 | 0.5341 | 0.5343 | 0.5342 | | | |
| 10 µg/mL | $S_0$ | 0.0258 | 0.0258 | 0.0259 | 0.0258 | 0.5084 | 42.1286 | |
| | $S_1$ | 0.4436 | 0.4435 | 0.4436 | 0.4436 | | | |
| 12.5 µg/mL | $S_0$ | 0.0218 | 0.0217 | 0.0219 | 0.0219 | 0.4217 | 51.9977 | |
| | $S_1$ | 0.3037 | 0.3037 | 0.3035 | 0.3037 | | | |
| 15 µg/mL | $S_0$ | 0.0198 | 0.0198 | 0.0198 | 0.2839 | 0.2839 | 67.6835 | |

**Table 5:** Antidiabetic activity of quercetin

| Concentration (µg/mL) | | Absorbance | | | Absorbance Average | $S_1$-$S_0$ | % Inhibition | $IC_{50}$ |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | | | |
| Blank (No Extract) | $S_1$ | 0.9171 | 0.9167 | 0.9154 | 0.9164 | | | |
| | $S_0$ | 0.0384 | 0.0376 | 0.0377 | 0.0379 | 0.8785 | 0 | |
| | $S_1$ | 0.6828 | 0.6825 | 0.6826 | 0.6826 | | | |
| 1 µg/mL | $S_0$ | 0.0319 | 0.0315 | 0.0314 | 0.0316 | 0.6510 | 25.8964 | |
| | $S_1$ | 0.5260 | 0.5261 | 0.5260 | 0.5260 | | 42.9595 | |
| 2 µg/mL | $S_0$ | 0.0249 | 0.0249 | 0.0249 | 0.0249 | 0.5011 | | |
| | $S_1$ | 0.3709 | 0.3708 | 0.3708 | 0.3708 | | 61.0700 | |
| 3 µg/mL | $S_0$ | 0.0289 | 0.0288 | 0.0288 | 0.0288 | 0.3490 | | |
| | $S_1$ | 0.2520 | 0.2518 | 0.2520 | 0.2519 | | 74.4337 | |
| 4 µg/mL | $S_0$ | 0.0274 | 0.0274 | 0.0272 | 0.0273 | 0.2246 | | 2.4850 |
| | $S_1$ | 0.0737 | 0.0737 | 0.0737 | 0.0737 | | | |
| 5 µg/mL | $S_0$ | 0.0364 | 0.0362 | 0.0362 | 0.0364 | 0.0124 | 95.5885 | |

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**Table 6:** Value $IC_{50}$

| Sample | $IC_{50}$ (µg/mL) |
|---|---|
| *P.emblica* fruit ethanol extract | 4.5833 |
| Acarbose | 9.6862 |
| Quercetin | 2.4850 |

## Conclusion

The present study showed that the ethanol extract of *P. emblica* fruit has anti-cholesterol and anti-diabetic activities. The cholesterol lowering ability of the extract was found to be higher than that of fenofibrate but lower than that of simvastatin. It alpha-glucosidase inhibitory effect was also higher than that of acarbose.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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