Trop J Nat Prod Res, April 2024; 8(4):6975-6980 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

HMG-CoA Reductase Inhibition Activity of Sea Pandan Leaves *(P. tectorius)*

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ARTICLE INFO

Article history:
Received 27 September 2023
Revised 12 April 2024
Accepted 23 April 2024
Published online 01 May 2024

ABSTRACT

Hypercholesterolemia is characterized by elevated blood cholesterol levels, exceeding the normal limit of 200 mg/mL. One effective approach to lower the levels is by inhibiting the HMG-CoA reductase enzyme. Therefore, this study aimed to examine HMG-CoA reductase inhibition activity, phytochemical screening, total phenolic content(TPC), and total flavonoid content(TFC) using natural materials such as *P.tectorius* leaves. The methods used included simplicia standardization. The samples were gradually macerated using n-hexane, ethyl acetate, and methanol. The results of the maceration obtained PH(Pandanus Hexane), PE(Pandanus Ethyl acetate), and PM(Pandanus Methanol) extracts. The PE was fractionated using vacuum liquid chromatography(VLC), the results are PEF1, PEF2, PEF3, PEF4, and PEF5. The anti-hypercholesterol test was conducted on HMG-CoA reductase enzyme inhibition activity, while TPC and TFC were assessed using the Folin-Ciocalteu and the colorimetric methods. The results of the water, total ash, acid-insoluble ash content, water-soluble, and ethanol-soluble extractive values of simplicia were (6.7, 8.8, 0.76, 6.6, and 5.9)%, respectively. The phytochemical screening of simplicia, extracts, and fractions indicated the presence of flavonoids, saponins, terpenoids, tannins, phenolics, and quinones. The extract that showed the highest value of TPC and TFC was PE(32.65 mg GAE/g dry extract and 5.59 QE/g dry extract, respectively), while the fraction that showed the highest value of TPC and TFC was PEF3(24.95 mg GAE/g dry extract and 4.84 QE/g dry extract, respectively). So, the research results showed that PE (IC_{50}=42.61 ppm) and PEF3 (IC_{50}=95.22 ppm) showed the highest inhibitory activity of HMG-CoA reductase compared to other extracts and suspected as potential anti-hypercholesterol agents.

***Keywords*:** Anti-hypercholesterol; HMG-CoA reductase; Sea Pandan Leaves (*P. tectorius);* Phytochemical screening; TPC; TFC

Introduction

Hypercholesterol is a significant risk factor for morbidity and mortality worldwide. This condition was characterized by elevated cholesterol levels in the blood, exceeding the normal limit of 200 mg/mL. Increased levels of LDL in blood vessels can contribute to the development of atherosclerosis.[1] Although anti-hypercholesterol drugs are widely available on the market, the majority are unsafe for long-term consumption due to their potential side effects on the digestive tract and liver.

One natural ingredient with great potential to lower cholesterol levels by inhibiting HMG CoA enzyme activity is sea pandan (*P. tectorius) P.* tectorius fruit has potential as a natural anti-hypercholesterol, but the potential of the leaves has not been widely reported. The HMG-CoA reductase enzyme is a rate-limiting enzyme that can catalyze the rate of mevalonate formation in cholesterol biosynthesis.[2]

P. tectorius belongs to the *Pandanaceae* family and is widespread across the Pacific region, including Indonesia (Irian Jaya, Java, and Maluku). The plant grows along the coast and has several benefits, including as a hypolipidemic, anti-inflammatory, anti-oxidant, and anti-bacterial agent.

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Citation: Astari JIR, Anam K, Aminin ALN, Farras F. HMG-CoA Reductase Inhibition Activity of Sea Pandan Leaves *(P. tectorius)*. Trop J Nat Prod Res. 2024; 8(4):6975-6980. https://doi.org/10.26538/tjnpr/v8i4.31

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

The fruit was reported to exhibit anti-hypercholesterol and anti-atherosclerotic effects by inhibiting HMG-CoA reductase enzyme activity. It also has great potential as a natural anti-hypercholesterolemia agent, but information on leaves is rare.[3]

One primary pharmacological agent for treating high cholesterol is HMG-CoA inhibitors, commonly known as statins. However, long-term use is associated with undesirable side effects such as myalgia, rhabdomyolysis, myopathy, hepatotoxicity, peripheral neuropathy, headache, diarrhea, and allergy. Statins competitively inhibit the HMG-CoA reductase enzyme. Statins bind to the active site of the enzyme and change its structure. This limits the tendency of the enzyme to bind its receptor, thereby reducing the activity.[4] Therefore, this study aimed to examine HMG-CoA reductase inhibition activity, phytochemical screening, as well as total phenolic (TPC), and total flavonoid content (TFC) of *P. tectorius* leaves.

Materials and Methods

The chemicals used were obtained from Merk Germany, including ethyl acetate, n-hexane, methanol, phytochemicals and TLC dyeing reagents, ethanol, amyl alcohol, ether, anhydrous acetic acid, concentrated sulfuric acid, 96% ethanol, iron(III) chloride 1%, Na_2SO_4, benzene, chloroform, butanol, dichloromethane, sulfuric acid, sodium hydroxide, aluminum chloride, n-hexane for analysis, methanol for analysis, benzene for analysis, diethyl ether for analysis, Folin-Ciocalteu, sodium carbonate, silica gel 60 H, TLC silica gel 60 F_{254}, Gallic acid, and quercetin are obtained from Sigma Germany, HMG-CoA Reductase Assay Kit (Sigma Aldrich, USA), Thermo fisher pipette, Spectrophotometer UV-Vis (Genesys 10S).

Plant Collection and Identification
P. tectorius leaves were collected from Pailus Beach, whose coordinate location is -6°29′54.88 north latitude and 110°41′21.79 east longitude, Jepara, Central Java, Indonesia, in October 2021, and was determined by the taxonomist (Mr. Rully Rahardian) with the herbarium number is 1b.2b.3b.4b.6b.7b.9b.11a…67a.68a at the ecology and biosystematics laboratory Diponegoro University, Central Java, Indonesia.

Sample Preparation (Simplicia Standardization)
The simplicia standardization was carried out to assess the water, total ash, acid-insoluble ash content, water-soluble extract content, and ethanol-soluble extract content.[5]

Phytochemical Screening
Phytochemical screening was conducted according to the Indonesian Materia Medika procedures.[5]

Extraction Method
Dried *P. tectorius* leaves were powdered, and 1 kg of powdered leaf was macerated using 7 Liter n-hexane, ethyl acetate, and methanol, sequentially. The maceration process was carried out for 24 hours. The filtrate was concentrated with a rotary evaporator at 50°C. *P. tectorius* leaves extracts were named hexane extract (PH), ethyl acetate extract (PE), and methanol extract (PM). The percentage yield (% w/w) was calculated using the following formula: [6]

$$\text{Yield of extract (\%)} = \frac{\text{extract weight (g)}}{\text{sample weight(g)}} \; x \; 100$$

Fractionation Method
The fractionation of the active extract used the vacuum liquid chromatography (VLC) method. 5 grams of active extract (PE) dissolved in 5 mL of methanol and thoroughly combined with 5 grams of silica gel. Subsequently, pour the dry silica that was saturated with the sample into the column and allow it to settle without a vacuum, using a column length-to-diameter ratio of about 8:1. Once all of the samples were added, a vacuum was applied and eluted with a stepwise gradient system consisting of n-hexane: ethyl acetate (100 : 0, 90 : 10, 80 : 20, 60 : 40, 40 : 60, 20 : 80, and 0 : 100) and ethyl acetate: methanol (80 : 20, 60 : 40, 40 : 60, 20 : 80, and 0 : 100) mL. Applied a vacuum once the solvent has been added to the top of the column. This process was repeated five times, and the fractions were collected in vials. All fractions were evaporated, weighed, and analyzed using the TLC method.[7]

Phytochemical Screening Analysis Method with TLC
Qualitative phytochemical screening analysis was performed using standard methods.[8] Silica gel G60 F_{254} was used as the stationary phase and activated by heating at 110°C for 15 minutes. Extracts and fractions of *P. tectorius* were applied to TLC plates using a capillary tube. At the same time, elution was carried out using dichloromethane-chloroform (7:3), n-hexane-ethyl acetate-diethyl ether (7:2:1), and dichloromethane-butanol (7:3) for PH, PE, and PM, respectively. Meanwhile, benzene-ethyl acetate (5.5:4.5) was used for PEF1, PEF2, PEF3, PEF4, and PEF5. The spots obtained were identified using specific spray dyeing reagents and under UV light at 254 nm and 366 nm.[9]

Total Phenolic Content (TPC) Assay Method
TPC of extracts and fractions were determined using the Folin-Ciocalteu method. Standard gallic acid curves were prepared with dilutions (0.02, 0.04, 0.06, 0.08, and 0.1 mg/ml) in methanol from a standard solution. Each of the dilutions (100 L) was mixed with 500 L water, and 1000 L Folin-Ciocalteu reagent, and allowed to stand for 6 minutes. Subsequently, 1 mL of 7.5% sodium carbonate and 500 L distilled water were added to the reaction mixture. The absorbance was recorded at 765 nm spectrophotometrically after 90 minutes and the same procedure was repeated for all fractions. TPC was calculated as gallic acid equivalents (mgGAE/g) and all experiments were performed in triplicate. All the samples were calculated using the formula: $C = c\frac{V}{m}$, where C: total phenolic content mg GAE/g dry extract,c: concentration of gallic acid obtained from the calibration curve in mg/mL, V: volume of extract in mL, and m: mass of extract in gram.[10]

Total Flavonoid Content (TFC) Assay Method
The TFC of the extracts and fractions was determined with the colorimetric method. The extracts were dissolved in a 10 mL methanol solution, then 1 mL extract solution was diluted with distilled water (10 mL) to obtain a concentration of 1 mg/mL. About 0.5 ml test sample was added with 1.5 mL methanol, 0.1 ml 10% $AlCl_3$, 0.1 m L, 1 M sodium acetate, and 2.8 mL distilled water. After 30 minutes of incubation, the absorbance was measured with a UV-Vis spectrophotometer. The TFC was expressed as quercetin equivalents using the linear equation based on the calibration curve. [10]

Inhibition of HMG-CoA Reductase Activity
The inhibitory activity of HMG-CoA reductase was determined with spectrophotometric measurements. The extracts and fraction were dissolved in methanol with serial concentration, as well as pravastatin as a positive control. Each extract was mixed with a reaction mixture containing assay buffer, NADPH, and HMG-CoA substrate solution, followed by the addition of HMG-CoA reductase. The mixture was incubated at 37°C and the absorbance was measured using UV-Vis spectrophotometry at 340 nm after 10 minutes. The IC_{50} value for the enzyme inhibition was then calculated. [11]

Result and Discussion

Standardization was performed on the *P. tectorius* leaves simplicia. The method follows the guidelines written in the Indonesian herbal famacopoeia (FHI). The parameters standardization test carried out consisted of water, total ash, acid-insoluble ash, water-soluble, and ethanol-soluble extracts and the results are presented in Table 1. According to Materia Medica Indonesia (1989), for the water content, total ash, acid-insoluble ash, water-soluble extract content, and ethanol-soluble extract content, the acceptable limits for these parameters are less than 8%, 9%, 1%, 7%, and 6%.[9]

Table 1: Standardization Results of *P. tectorius* Leaves Samples

Sample Characterization	**Value (%)**	**SNI Standard (%)** (Materia Medica Indonesia (1989))
Water	6.7	< 8
Total Ash content	8.8	< 9
Acid Insoluble Ash	0.76	< 1
Water Soluble Extract	6.6	< 7
Ethanol Soluble Extract	5.9	< 6

Based on the results (Table 1) obtained, it can be concluded that the water content obtained from Simplicia meets the quality requirements. The determination of water content is also related to the purity of simplicia. The high water content that high causes the growth of microbes, which will reduce the stability of simplicia. The higher the total ash content obtained, the higher the mineral content contained in the simplicia, while the acid-insoluble ash content reflects the presence of acid-insoluble mineral or metal contamination in a product. The high content of ash insoluble in acid indicates the presence of silicate content originating from soil or sand and the metallic elements silver, lead, and mercury.[9] The water-soluble extract content has a higher value compared to the ethanol-soluble extract content. This means that secondary metabolite compounds that are polar and semi-polar will dissolve more easily in water compared to 96% ethanol. [9]

Based on the results of the standardization of the *P. tectorius* leaves simplicia, it was stated that the simplicia could be subjected to the next test, namely phytochemical screening. The principle of phytochemical screening is the analysis of chemical groups in plants through specific tests with the addition of reagents that will give a certain colour change to secondary metabolites. These results are in line with a previous study stating that *P. tectorius* leaves have secondary metabolites in the form of saponins, phenolics, terpenoids, and flavonoids but do not contain alkaloids.

The next step was the extraction of *P. tectorius* leaves was achieved using a multilevel maceration method with n-hexane, ethyl acetate, and methanol as solvents. The separation of bioactive compounds from the extracts was based on differences in polarity.[12] Based on the maceration process, the results obtained were PH (5.34 g); PE (7.58 g), and PM (4.08 g) extracts. Then, the percentage yield of *P.tectorius* leaves extract could be calculated and each was 1.84% (PH); 1.32% (PE), and 2.45% (PM).

Based on the extraction results, three extracts were obtained, namely PH, PE, and PM. From these three extracts, to ascertain the chemical composition of the *P. tectorius* leaves extracts, thin-layer chromatography was used, yielding the spot profile of each sample. The identification of flavonoids, saponins, terpenoids, tannins, phenolics, and quinones was determined using spray reagents (universal and specific). This analysis evaluated PH, PE, and PM, and the results are presented in Figure 1. The results of separation identified under UV light (254 nm and 366 nm).

Based on Figure 1(a), PH contains flavonoids with the formation of three separate brown spots on the TLC plate, and the RF values for each spot are 0.58, 0.29, and 0.26. Meanwhile, PE and PM show a blue-white color with an RF value of 0.36 in PE, which indicates the presence of catechins, and 0.1 in PM, which indicates the presence of flavonols.[13]

As shown in Figure 1(b), PH extract had stain 2 (blue) with an Rf value of 0.27 on the TLC plate, indicating the presence of tannins, while stains 1 and 3 had values of 0.55 and 0.24, representing phenol. Meanwhile, PE and PM formed spots and a dot, respectively, with Rf values of 0.63 and 0.78. Phenolic identification was confirmed after spraying with $FeCl_3$, followed by the formation of a reddish-brown color. [13] Based on the results, all extracts were found to contain phenolic compounds.

Figure 1(c) shows that PE and PM had red spots after being sprayed using NaOH, with RF-values of 0.36, 0.63, and 0.76 (for PE) as well as 0.8 (for PM). PH did not show any spots, but after spraying NaOH or KOH in PM, the color change shifted from yellow and orange to red, purple, green, or purple, indicating the presence of quinones.[14] Figure 1(d) shows the identification of terpenoids and steroids, with the Liebermann-Burchard spray reagent producing a green color on PH and PM, while PE produced a red color on the TLC plate. Rf values of PH were 0.36, 0.49, and 0.58, while those of PE and PM were 0.24, 0.36, 0.55, and 0.4, respectively. The green color on the TLC plate for PH and PM indicated the presence of steroids. Moreover, the red color on PH and PE plates represented terpenoids.[13] Based on TLC analysis, all leaves extracts were found to contain flavonoids, phenolics, quinones, and steroids.

Based on the results of phytochemical screening using TLC of the three extracts (PE, PH, and PM), the next test to determine anti-hypercholesterol activity was the HMG-CoA reductase enzyme inhibition assay. The results of anti-hypercholesterol activity are shown in Figure 6, where the IC_{50} values are PH (269.03 ppm), PE (42.61 ppm), and PM (389.11 ppm).

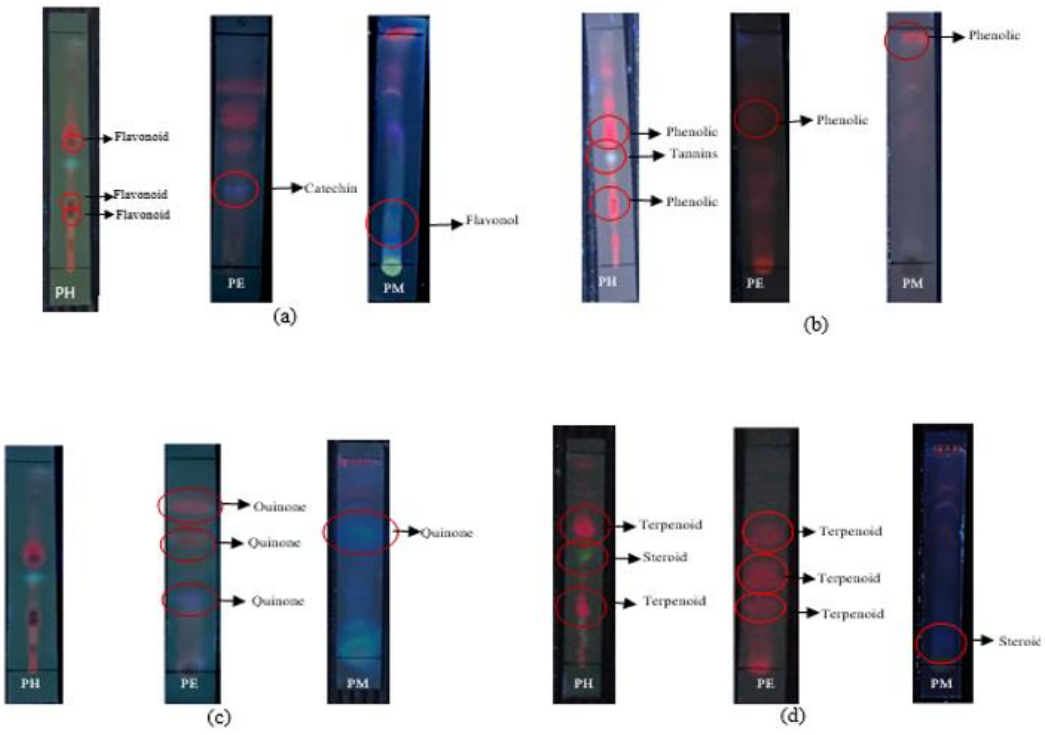

Figure 1: TLC analysis of *P. tectorius* leaves extracts (PH, PE, and PM). Identified with the spray reagent in (a). $AlCl_3$; (b). $FeCl_3$; (c). NaOH; (d). Liebermann-Buchard. Identified under a UV lamp at 365 nm.

The strongest extract is the PE extract, because the lower the IC_{50} value, the greater the activity of the extract to inhibit the HMG-CoA reductase enzyme. PE extract is semi-polar, so compounds with the ability to inhibit HMG-CoA reductase will react more strongly to extracts that are semi-polar, they can act as the strongest inhibitor of the activity of the HMG-CoA reductase enzyme. The bioactive compounds contained in the extract also influence the inhibition of the HMG-CoA reductase enzyme. The presence of flavonoids and phenolic compounds has been reported to inhibit HMG-CoA reductase.[15]

Based on inhibition of HMG-CoA reductase enzyme assay, it was found that the active extract of *P. tectorius* leaves is PE and then fractionated using the VLC method with a gradient eluent composed of n-hexane–ethyl acetate (100:0, 80:20, 60:40, 40:60, 20:80, 0: 100) mL and ethyl acetate – methanol (100:0, 80:20, 60:40, 40:60, 20:80, 0:100) mL was used in this process. Fractions were obtained and identified by TLC. Fractions having the same chromatogram points were combined to obtain PEF1, PEF2, PEF3, PEF4, and PEF5.

Based on the fractionation results, to ascertain the chemical composition of the *P. tectorius* leaves fractions, thin-layer chromatography was used, yielding the spot profile of each sample. This analysis evaluated PEF1, PEF2, PEF3, PEF4, and PEF5, and the results are presented in Figure 2. Based on the results, these fractions contained phenolic compounds (figure 2a) with RF values of PEF1, PEF3, PEF4, and PEF5 were 0.94, 0.07, 0.07, and 0.04, respectively, while PEF2 formed 2 spots with RF-values of 0.94 and 0.78, then contained flavonoids (Figure 2b) with RF-values of PEF 1, PEF 4, and PEF 5 were 0.92, 0.96, 0.18, and 0.09, while PEF2 and PEF3 formed a dark mauve color with the same RF-values of 0.78, besides that it also contains quinones (Figure 2c), where the RF values of PEF1, PEF2, PEF3, PEF4, and PEF5 were 0.84, 0.73, 0.93, 0.93, and 0.05, respectively and contained terpenoids (Figure 2d), where the RF values of PEF1 and PEF3 were 0.8 and 0.52, a blue color at two PEF2 points, and a red color on PEF4 and PEF5 on TLC plate.[13]

The results of the fractionation obtained an active fraction, and using it for the HMG-CoA reductase enzyme inhibition assay was carried out. Before carrying out the HMG-CoA reductase enzyme inhibition assay, the TFC and TPC processes were continued on the PH, PE, and PM extracts and the PEF1, PEF2, PEF3, PEF4, and PEF5 fractions. The extracts and fractions were first assessed using the Folin-Ciocalteu reagent and subsequently analyzed with a UV-Vis spectrophotometer. The phenolic compounds reacted with the Folin-Ciocalteu reagent and formed a blue complex with varying color intensities. The standard solution used was gallic acid, a stable and simple phenolic compound derived from hydroxybenzoic acid.[13] The measurement results were plotted into a calibration curve with the regression equation y = 6.8214x–0.0099 and an Rf value of 0.9987.

TPC was determined by the Folin–Ciocalteu colorimetric method using gallic acid (GAE) as standard, and various concentrations of the extract solutions were measured at 765 nm. The total phenolic content in the *P. tectorius* leaves extract and fraction was calculated using a linear regression equation. Based on the analysis results, TPC in the extract and fractions of *P. tectorius* leaves was 23.92 ± 0.043 and 19.63 ± 0.052 mg GAE/g, respectively. This indicated that every gram of the extract was equivalent to 23.92 mg and 19.63 mg of gallic acid (Tables 2 and Figure 3).

Table 2 shows that the highest TPC was found in PE at 32.65 mg GAE/g extract, while the yield for PEF3 was 24.95 mg GAE/g extract as depicted in Figure 3. Therefore, PE extract and PEF3 fraction were suspected as potential anti-hypercholesterol agents. TFC was determined using a method where a complex was formed between aluminum chloride and the keto group on the C-4 atom as well as the hydroxy group on the neighboring C-3 or C-5 atoms of the flavones and flavonol groups. Quercetin was used as a standard for determining flavonoid content because it belongs to the flavonol group, which has a keto group on the C-4 atom and a hydroxyl group on the neighboring atoms C-3 and C-5.[10]

The absorbance measurements were carried out at several concentrations of 0, 20, 40, 60, 80, and 100 ppm, resulting in a linear relationship between absorbance and concentration, with an R-value of 0.9992. From the calculation results, the intercept was determined to be -0.00395 and the slope value was 8.725, culminating in the standard curve equation of y = 8.7257x -0.004.

While TFC of *P. tectorius* leaves extracts and fractions was measured at 3.55 ± 0.012 mg QE/g extract (Figure 4), and 3.50 ± 0.0058 mg QE/g extract (Figure 5), this means that each gram of extract is equivalent to 3.02 mg of quercetin. Figure 4 and Figure 5 show that PE had the highest TFC of 5.59 mg QE/g extract, followed by PEF3 at 4.84 mg GAE/g extract. Therefore, PE extract and PEF3 fraction were suspected as potential anti-hypercholesterol agents. To test further, inhibition of the activity of the HMG-CoA reductase enzyme was carried out on the fractions (PEF1, PEF2, PEF3, PEF4, and PEF5).

Table 2: Total Phenolic Content of *P. tectorius* Leaves Extracts

Sample	Total Phenolic Content (mg GAE/g extract)
PH	17.97 ± 0.026
PE	32.65 ± 0.052
PM	21.14 ± 0.052
TPC ± SD	23.92 ±0.043

Note: PH=Pandanus Hexane Extract, PE=Pandanus Ethyl Acetate Extract,
PM=Pandanus Methanol Extract

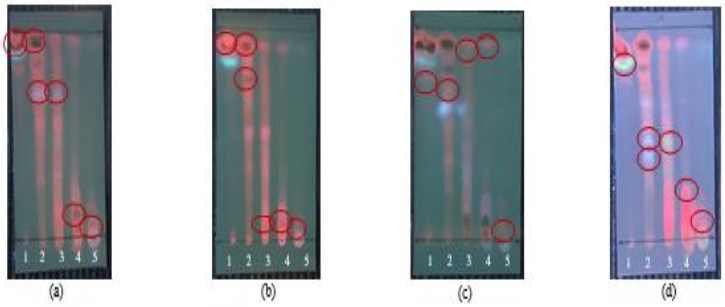

Figure 2: TLC analysis of *P. tectorius* leaves fractions (PEF1 to PEF5 from left to right). Identified with spray reagent (a) and $AlCl_3$; (b). $FeCl_3$; (c). NaOH; (d). Liebermann-Buchard. Identified under a UV lamp at 365 nm.

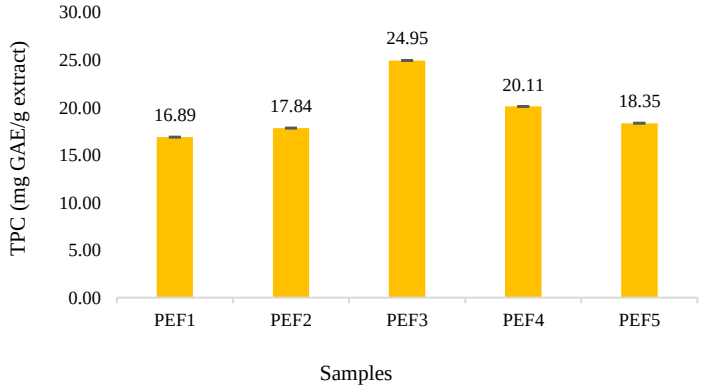

Figure 3 : Total Phenolic Content of *P. tectorius* Leaves Fractions
PEF1= Pandanus Ethyl Acetate Extract Fraction 1, PEF2= Pandanus Ethyl Acetate Extract Fraction 2, PEF3= Pandanus Ethyl Acetate Extract Fraction 3, PEF4= Pandanus Ethyl Acetate Extract Fraction 4, PEF 5= Pandanus Ethyl Acetate Extract Fraction 5

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

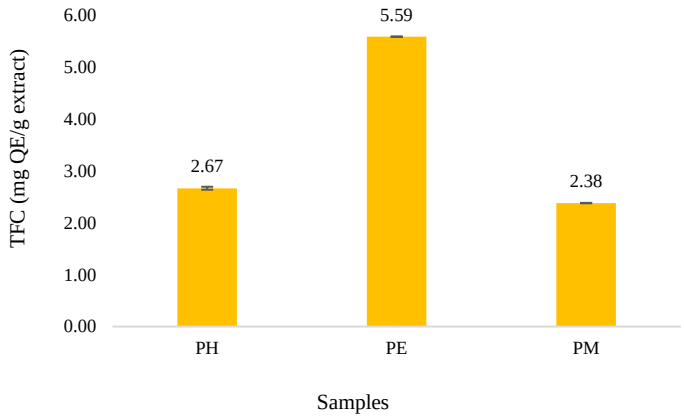

Figure 4: Total Flavonoid Content of *P. tectorius* Leaves Extracts
PH=Pandanus Hexane Extract, PE=Pandanus Ethyl Acetate Extract, PM=Pandanus Methanol Extract

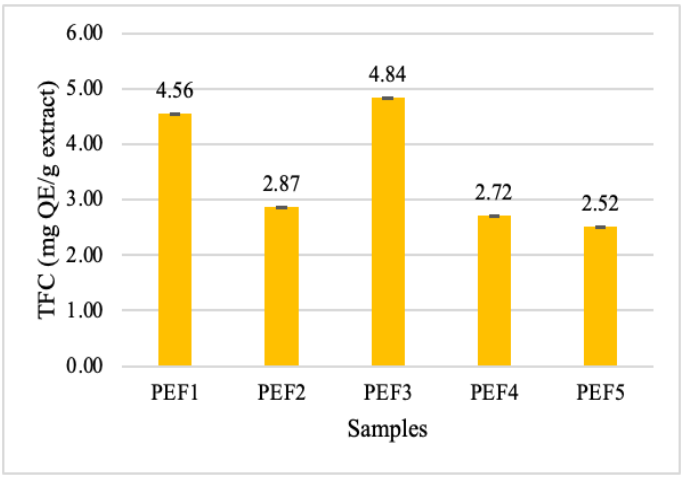

Figure 5: Total Flavonoid Content of *P. tectorius* Leaves Fractions
PEF1= Pandanus Ethyl Acetate Extract Fraction 1, PEF2= Pandanus Ethyl Acetate Extract Fraction 2, PEF3= Pandanus Ethyl Acetate Extract Fraction 3, PEF4= Pandanus Ethyl Acetate Extract Fraction 4, PEF 5= Pandanus Ethyl Acetate Extract Fraction 5.

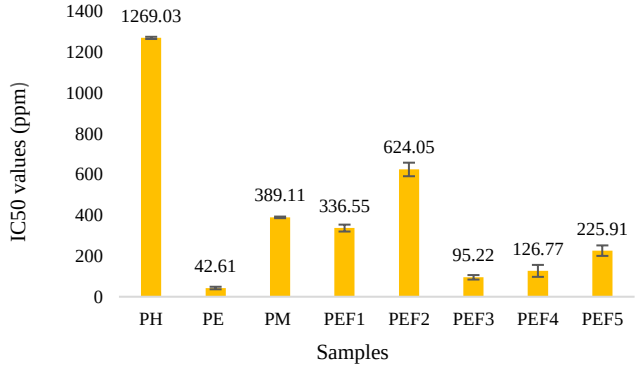

Figure 6: Inhibitors of HMG-CoA Reductase Activity from *P. tectorius* Leaves Extracts and Fractions
PH=Pandanus Hexane Extract, PE=Pandanus Ethyl Acetate Extract, PM=Pandanus Methanol Extract, PEF1= Pandanus Ethyl Acetate Extract Fraction 1, PEF2= Pandanus Ethyl Acetate Extract Fraction 2, PEF3= Pandanus Ethyl Acetate Extract Fraction 3, PEF4= Pandanus Ethyl Acetate Extract Fraction 4, PEF 5= Pandanus Ethyl Acetate Extract Fraction 5

Based on the results of HMG-CoA reductase enzyme activity inhibitors, the IC_{50} values for the fractions are presented in Figure 6. The PEF3 fractions showed that they exhibited the strongest activity (95.22 ppm), followed by PEF4 (126.77 ppm), PEF5 (225.91 ppm), PEF1 (336.55 ppm), and PEF2 (624.05 ppm). The lower IC_{50} values indicate greater activity of the extract and fraction in inhibiting the HMG-CoA reductase enzyme. Thus, the potential of *P. tectorius leaves* fractions to inhibit HMG-CoA reductase is lower when compared to pravastatin (positive control). This may occur because the sample is still a crude extract that contains various types of compounds that can influence the inhibitory reaction. However, PEF3 is the most powerful in inhibiting the HMG-CoA reductase enzyme activity.[16]

Conclusion

In conclusion, the phytochemical screening results for Simplicia, extracts, and fractions of *P. tectorius* leaves showed the presence of flavonoids, saponins, terpenoids, tannins, phenolics, and quinones. The strongest inhibitors of the HMG-CoA reductase enzyme were PE extract (IC_{50} = 42.61 ppm) and PEF3 fraction (IC_{50} = 95.22 ppm). Furthermore, the analysis results showed that PE and PEF3, derived from ethyl acetate (a semi-polar) solvent, had a higher TFC value than TPC. This indicates that the mechanism of HMG-CoA reductase enzyme inhibition is mainly elicited by compounds belonging to the flavonoid group. The high flavonoid content presumably inhibited the enzyme by binding to its active site, thereby preventing catalytic activity.

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.

Acknowledgements

The authors are grateful to the Directorate of Research and Community Services, Ministry of Research and Technology of Indonesia for funding this study through the Higher Education Fundamental Research Scheme.

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