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# Antihypercholesterol activity of ethanol extract of *Bauhinia hullettii Prain* and its secondary metabolites

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**ABSTRACT**

*In vivoantihypercholesterol assay of ethanol extract of Bauhinia hullettii Prainshowed real impact in decreasingtotal cholesterol level and the LDL (low density lipid)in male Wistar strainrats (Rattus norvegicus)significantly. This research indicated250 mg/kgbb of ethanol extract is the most effective dose to decreasetotal cholesterol level and LDL. The separation of secondary metabolites in this plant yielded one phenolic compound, 3,4,5-trihydroxybenzoate acid (1) and two flavanones, 5,7,3',5'-tetrahydroxyflavanone (2) and 5,7,4'-trihydroxy-6-methylflavanone (3). These three compounds were reported for the first time in this plant.*

**Keywords:** *B.hullettii* Prain, antihypercholesterol, flavonoid, phenolic

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## INTRODUCTION

Hypercholesterol is the increasing of cholesterol level in blood. Hypercholesterol is reported to cause coronary heart disease (CHD)with 4.4 millions or 18% case around the world [1]. Hypercholesterol and its relevancy with CHDstimulates the development of blood cholesterol lowering or antihypercholesterol drugs.

Antioxidative compounds such as flavonoids and phenolics are knowing as the potential compounds to decreaseblood cholesterol. *Bauhinia*is declared to have flavonoids, coumarines, tannins, terpenoids [2], cyanoglucoside [3], steroid [4], quinnone [5], and phenolic [6]. Flavonoidswidely isolated from *B. Purpurea*are bis[3'4'-dihydroxy-6-metoxy-7,8-furano-5',6'-monomethyl-alliloksi]-5-C-5-biflavonil and 4'-hydroxy-7-methyl 3-C-α-L-ramnopyranosyl-5-C-5-(4'-hydroxy-7-methyl-3-C-α-D-gluco-pyranosyl) biflavonoid [7], quercetin-3-O-rutinosidaandquercetinfrom*Bauhinia monandra* [8], 6C,7-O-dimethylaromadendrin and ploretin from stem bark of*B.excelsa* [9], 3,5,7,3',5'-pentahydroxyflavanoneol-3-O-α-L-rhamnopyranosideandquercetin from stem bark of *B.strychnifolia* [10], quercetin-3-O-α-arabinose, quercetin, quercetin-3-O-α-rhamnoside, quercetin-3-O-β-glucosideandquercetin-3-O-β-galactopyranosefrom leaves of *B. Longifolia*[11].

*Bauhinia* is a genus in Leguminoceae family containing 300 species [12]. This genus has been known because its biological activities like anticancer [13], antioxidant [14-16], hypoglycemia [17], hyperlipidemia [18], antivirus [11], antiinflammation [3], antimicrobial [19],andanalgesic [20].*Bauhinia hullettii* Prain with traditional name "tapak kuda" is one of the *Bauhinia* species found widely in Indonesia. Literature search indicates no phytochemical screening has been reported.

On the previous report, 5,7,3',5'-tetrahydroxyflavanone (2) and 5,7,4'-trihydroxy-6-methylflavanone (3) [21] have been isolated. On this paper, it will be determinedthe structure of compound (1) based on spectroscopy data including UV, IR, and NMRincluded 2-D NMR. Antihypercholesterol effect assay from ethanol extract treated to male rats (*Rattus norvegicus)* will be reported.

## MATERIALS AND METHODS

### 2.1 Materials and Tools

The materials used were3-monthmale *Wistar strain* rats taken from Faculty of Pharmacy of Andalas University, Padang and *Bauhinia hullettii* Prain leaves from Hutan lindung PT. Bukit Asam TanjungEnim, South Sumatra in January 2016. Ethanol 96%, fodder, cholesterol fodder (cow fat and fodder), ether, cholesterol reagent kit (cholesterol SL), triglyceride (triglycerides mono SL New), HDL (cholesterol HDL SL), methanol, hexane, ethylacetate, dichloromethane, silica gel Merck 60 $GF_{254,}$ silika gel Merck 60 (230-400 mesh), silika gel Merck 60 G (70-230 Mesh) and thin layer chromatography (TLC) Merck 60 $GF_{254}$, 0.25 mm. Tools used were analytical balance, micro pipettes, sonde pipettes, disposable syringe,animal cage, EDTA tube, microtube, capilar tube, *cholesterol test strips*,centrifuges, *Clinical Chemistry Auto Analyzer* tipe *Selectra E*, rotary evaporator, glassware, Fisher John melting point tool, spektrofotometer UV-Vis Shimadzu, PerkinElmer spectrum one FT-IR, spektrometer JEOL JNM ECA-500, 500 MHz ($^1$H) and 125 MHz ($^{13}$C)

### 2.2Procedure

**Extraction and Isolation**

*B. hulllettii* Prain (1.4 kg) leaves were macerated with 3 repetitions with n-hexane, dichloromethane, and ethylacetate to yield fractionn-hexane (28.64g), dichloromethane (39.10 g) andethylacetate (21.32 g). Ethylacetate fraction was eluted with LVC using *n*-hexane-ethylacetate with increasing polarity from 10:0 to ethylacetate 100% to afford 16 fractions (A-P). Fraction I (1.833 g) was separated with GCC using n-hexane,ethylacetateandacetone to afford 14 fractions ($I_1$ – $I_{14}$).Fraction $I_9$(187 mg) was then purified with dichloromethane to yield non-dissolved crystal ( greenish white). It was washed with n-hexane-ethylacetate (1:1) to yield compound 1 (20 mg).Isolation process of compound 2 and 3 have been completely reported on the previous publication [21].

***In vivo* antihypercholesterol assay**

The research was initiated by making ethanol extract of *B.hullettii* Prain leaves (*B.hullettii* Prain). The extract was taken with maceration method of 1 kg of air-dried leaves using ethanol 96%. Maceration remained for three days with three repetitions. The extract was evaporated to yield ethanol crude extract (145 g). The crude was then dissolved with aquadest to make the sample liquid at doses 50, 150 and 250 mg/kgbb.

The next stage was to place 20 rats into 5 groups. The first group as negative control (A), group 2 to 5 as tested groups (B-E). The rats were fed with standar fodder and *ad libitum*water.

The four tested groups (B-E) were fed with high cholesterol diet fodder (the mixture of fodder:cow fat=70:30). The tested rats were assigned as total cholesterol level, triglyceride, HDL, LDL after treating with high cholesterol diet for 14 days.

The last stage, the three tested rats groups were treated with 50, 150 and 250 mg/kgbb for group C, D, and E respectively. The treatment lasted for 28 days. Total cholesterol level, triglyceride, HDL, and LDL determination were measured on day 14 and 28.

**Spectral Data**

3,4,5-trihydroxybenzoate (1). M.p. 246-247 °C; $FeCl_3$ test: (+); UV (MeOH) $\lambda_{max}$ nm: 265.4; IR $\nu_{maks}$ $cm^{-1}$: 3254, 1702, 1614, 1541, 1246;$^1$H NMR (500 MHz, Acetone-*d6)δ ppm*: 7.15 (2H, s); $^{13}$C NMR (125 MHz, Acetone-*d6δ ppm*: 167.78 (COOH), 146.02 (C-3 dan C-5), 138.64 (C-4), 122.12 (C-1), 110.09 (C-2 and C-6).

*5,7,3′,5′-tetrahydroxyflavanone* (2). M.p. 263–265 °C; $FeCl_3$ test: (+); UV (MeOH) $\lambda_{max}$ nm: 328 (bh), 288 and203; UV (MeOH+ NaOH) $\lambda_{max}$ nm: 325, 265, 212; UV (MeOH+$AlCl_3$) $\lambda_{max}$ nm: 368 (bh), 347, 305, 264, 205; UV (MeOH+$AlCl_3$+HCl) $\lambda_{max}$ nm: 368 (bh), 348, 307, 265, 206; IR (KBr) $\nu_{maks}$ $cm^{-1}$: 3365, 2920, 1635, 1602, 1450, 1257, 1161;$^1$H NMR (500 MHz, Methanol-d3)*δ ppm*: 2.69 (1H, dd, *J*=17 Hz, 3.0 Hz, H-3a), 3.07 (1H, dd, *J*=17 Hz, 13 Hz, H-3b); 5.28 (1H, dd, *J*=13 Hz, 3.0 Hz, H-2), 5.94 (1H, d, J= 2 Hz, H-8), 5.95 (1H, d, J= 2 Hz, H-6), 6.78 (2H, s, H-2′ and H-6′), 6.91 (1H, s, H-4′); $^{13}$C NMR (125 MHz, Methanol-d3*δ ppm*: 197.84 (C4), 168.61 (C7), 164.95 (C9), 165.57 (C5), 147.00 (C3′), 146.61 (C5′), 131.86 (C1′), 119.33 (C4′), 116.32 (C2′), 114.78 (C6′), 103.40 (C10), 97.11 (C6), 96.26 (C8), 80.59 (C2), and 44.19 (C3).

5,7,4’- trihydroxy-6-methylflavanone (3). M.p. 263–265 °C; $FeCl_3$ test: (+); UV (MeOH) $\lambda_{max}$ nm: 365 (bh), 291, and 212; IR $\nu_{maks}$ $cm^{-1}$: 3336, 2916, 1614, 1601, 1462, 1262, 1148;$^1$H NMR (500 MHz, Acetone,-d6)*δ ppm*: 1.98 (3H, s, $CH_3$), 2.81 (1H, dd, *J*=17 Hz, 3.0 Hz, H-3a), 3.08 (1H, dd, *J*=17 Hz, 13 Hz, H-3b); 5.76 (1H, dd, *J*=13 Hz, 3.0 Hz, H-2), 6.09 (1H, d, s, H-8), 6.93 (1H, d, 7.8 Hz, H-3′), 6.95 (1H, d, 7.8 Hz, H-5’), 7.21 (1H, dd, 7.8 Hz, 1.3

Hz, H-6′), 7.52 (1H, dd, 7.8 Hz, 1.3 Hz, H-2′); $^{13}$C NMR (125 MHz, Methanol-d3$\delta$ *ppm*: 197.46 (C4), 165.00 (C7), 162.29 (C5), 162.13 (C9), 154.81 (C4′), 130.22 (C6′), 127.75 (C2′), 126.53 (C1′), 120.74 (C5′), 116.29 (C3′), 104.73 (C6), 103.05 (C10), 95.20 (C8), 75.39 (C2),42.76 (C3), and 7.12 ($CH_3$).

## RESULTS AND DISCUSSION

### Antihypercholesterol Activity

The result showing cholesterol high diet by giving cow fat fodder involved the increasing of total cholesterol concentration and LDL of tested rat blood. Total cholesterol concentration average and LDL of the four groups reached 147-152 mg/dl and 79-84 mg/dl. Cholesterol giving in high cholesterol diet caused cholesterol in cow fat is taken by kilomicron to liver. This affected the increasing of cholesterol concentration in liver. Some cholesterol was carried by VLDL and underwent hydrolysis by lipoprotein lipase to produce LDL [22]. This is the underlying the increasing of LDL level after high cholesterol diet.

Antihypercholesterol effecf of ethanol extract of tapak kuda leaves can be seen from its ability to decrease total cholesterol level and LDL of hipercholesterol rats blood. The decreasing of cholesterol concentration started to begin on week 2 , and at the end of the treatment (week 4), the total cholesterol concentration of rat blood decreased 12%, 18%, and 22.2% for group C, D, and E respectively. Based on statistical evaluation, total cholesterol concentration changing of ethanol extract of *B. Hullettii*Prain leaves was effective to decrease total cholesterol level for real. LDL concentration of rat blood of group C, D, and E was also lower as19.5%, 36.2%, and 48.6%. It can be concluded that ethanol extract of tapak kuda leaves is effective to decrease the LDL level.

**Table 1: Total Cholesterol Level Average and LDL of Rat Blood Serum After Treatment**

| Group | Total cholesterol (mg/dl) | | | LDL (mg/dl) | | |
|---|---|---|---|---|---|---|
| | Early | Middle | End | Early | Middle | End |
| A | 70.50 ± 4.358 | 70 ± 2.449 | 67.00 ± 2.708 | 11.75 ± 2.217 | 13.75 ± 2.629 | 11.75 ± 2.217 |
| B | 149.75 ± 10.812 | 156.25 ± 10.144 | 154.25 ± 11.899 | 84.00 ± 12.247 | 90.75 ± 11.757 | 89.50 ± 14.387 |
| C | 152.00 ± 5.598 | 138.25 ± 2.5 | 133.75 ± 3.304 | 79.50 ± 8.103 | 69.25 ± 7.135 | 64.00 ± 5.090 |
| D | 147.75 ± 7.228 | 133 ± 3.741 | 120.50 ± 7.416 | 79.50 ± 6.240 | 67.25 ± 5.67 | 50.75 ± 8.220 |
| E | 152.00 ± 9.486 | 133.75 ± 9.639 | 118.25 ± 13.889 | 81.75 ± 10.468 | 69.5 ± 7.724 | 42.00 ± 15.491 |

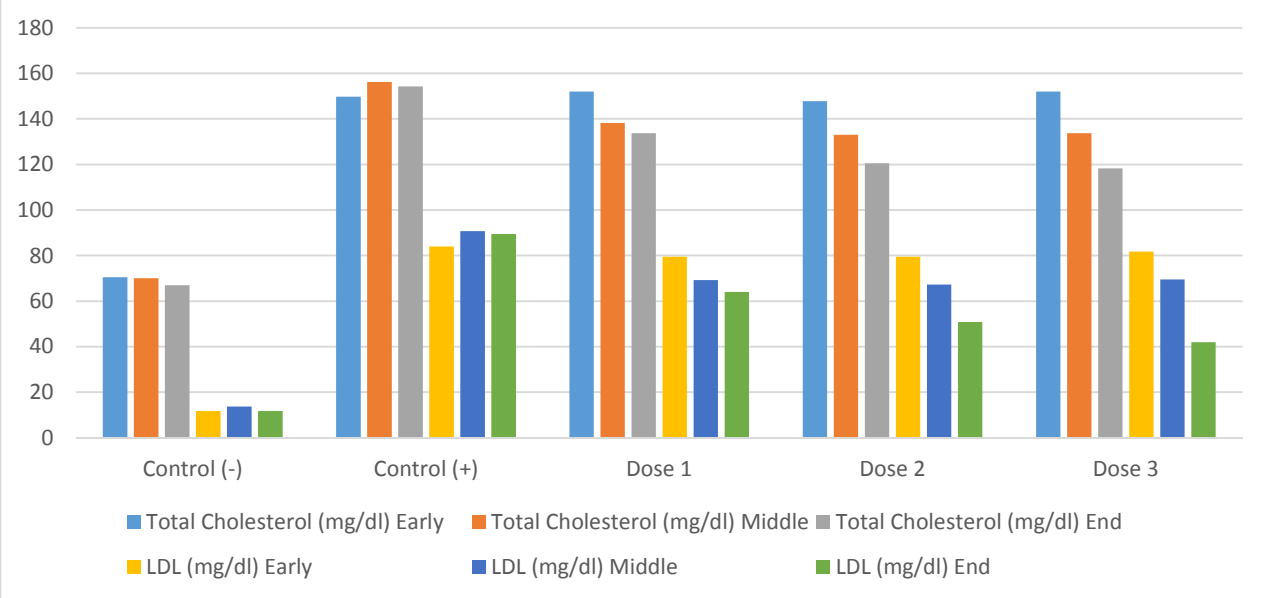



**Figure 1. Total Cholesterol Level and LDL of Rat Blood Serum After Treatment**

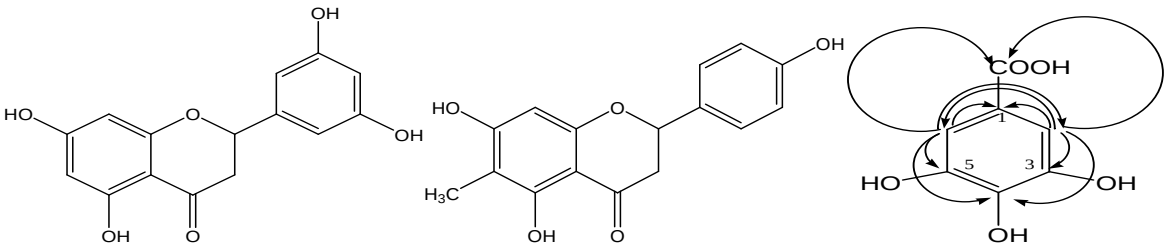
### Structure Analysis

Maceration process of air-dried leaves of *B. hullettii*Prain (1.4 kg) using n-hexane, dichloromethane, andethylacetate yielded fraction n-hexane 28.64 g, dichloromethane 39.1,andethylacetate21.32 g. Ethylacetate fraction was purified with vacuum chromatography to get 16 fractions (A-P). More purification on fraction I yielded compound 1. Compound 2 and 3 have been isolated from ethyl acetate fraction of *B. hullettii* Prain stem bark. Isolation and structure determination of compound 2 and 3 have been reported previously [21].

UV spectra of compound 1 exhibited absorption at $\lambda_{max}$265.4 nm, appropriate for aromatic ring chromophore. IR spectra showed some absorptions suitable for hydroxyl (3254 $cm^{-1}$), carbonyl (1702 $cm^{-1}$), C=C aromatic (1614, 1541 $cm^{-1}$), and C-O axyaril (1246 $cm^{-1}$) group. The next analysis for compound 1 was taken from $^{1}$H NMR,$^{13}$C NMR, 2D NMR.

$^{1}$H NMR spectra displayed one peak in aromatic region at $\delta_H$ 7.15ppm (2H,s). $^{13}$C NMR data analysis displayed some signals appropriate for 7 carbon atoms, included one carbonyl carbon (δ 167.78 ppm, C=O), and 6 aromatic carbons (δ 110.09-167.78 ppm).Trisubstitution of aromatic ring was proved with signals at 146.02 ppm and138.64ppm. A signal at 146.02 ppm was a signal for a meta carbon against COOH group (C-3 and C-5), while a signal at 138.64 ppm was compatible for para carbon against COOH group (C-4). A signal at 110.09 ppm has been consistent for two-symmetric carbons (C2 and C-6).

HMBC spectral analysis of compound 1 exhibited two-bond correlation of proton H-2 $\delta_H$ 7.15 ppm with C-1 and C-3 and three-bond correlation with C-6, C-4, and COOH. Proton H-6 $\delta_H$ 7.15 ppm has correlation five-neighbor carbon atoms through two and three-bond correlations (Figure 2). $^{1}$H NMR,$^{13}$C NMR, and 2D NMR spectral analysis support compound 1 as 3,4,5-trihydroxybenzoate. More information for structure determination of compound 1 is taken from comparison with spectra data in literature [23].




**(1)** **(2)** **(3)**

**Figure 2. The Structures of Isolated Compounds from Stem Bark and Leaves of *B. hullettii* Prain**

## CONCLUSION

Ethanol extract of *B. hullettii* Prain leaves has an effective effect to decrease the total cholesterol level and LDL in tested animals. The effective dose of ethanol extract of *B. hullettii* Prain is 250 mg/kgbb, where it can decrease total cholesterol in the amount of 22.2% and LDL 48.6%.

5,7,3',5'-tetrahydroxyflavanon, 5,7,4'-trihydroxy-6-methylflavanon, and 3,4,5-trihydroxybenzoate acid have been isolated from ethyl acetate fraction of stem bark and leaves of *Bauhinia hullettii* Prain. These compounds are reported for the first time in this plant.

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