Isolation and identification of a flavonoid compound and *in vivo* lipid-lowering properties of *Imperata cylindrica*

SITI KHAERUNNISA[1], NANIK SITI AMINAH[2], ALFINDA NOVI KRISTANTI[2], SUTJI KUSWARINI[1], CITRAWATI DYAH KENCONO WUNGU[1], SOETJIPTO SOETJIPTO[1] and SUHARTATI SUHARTATI[3]

[1]Department of Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java 60132; [2]Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya, East Java 60115; [3]Department of Medical Biochemistry, Faculty of Medicine Universitas Wijaya Kusuma Surabaya, East Java 60225, Indonesia

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Abstract. Cogon grass (*Imperata cylindrica*/*I. cylindrica*) of the Gramineae family is found abundantly in nature, and the roots of this plant possess several beneficial biological properties. The present study aimed to isolate and identify flavonoid compounds from cogon grass roots and examine their potential as hypocholesterolemic agents. The flavonoid compound was isolated using a maceration method, followed by gravity column chromatography until a pure compound was obtained. The molecular structure of the isolated compound was determined using ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR spectroscopy. An *in vivo* lipid-lowering test used a randomized post-test only control group experimental design in rats with hypercholesterolemia. The animals were divided into four groups: K0, negative control; K1, positive control; K2, ethanol extract treated group; and K3, ethyl acetate fraction treated group, and the lipid profiles were examined at the end of the study. The isolated compound, 7,3',5'-trimethoxyflavonol, was collected in yellow powder form; was shown to be a flavonoids and was comprised of 18 carbon atoms and 16 hydrogen atoms. *In vivo* tests demonstrated that 15 mg/200 g body weight (BW) of an ethanol extract significantly lowered total cholesterol levels (P=0.001) but did not lower low-density lipoprotein (LDL) (P=0.109) and high-density lipoprotein (HDL) levels (P=0.003). The fraction of ethyl acetate administered at 15 mg/200 g BW was capable of lowering the total cholesterol levels significantly (P=0.002) and lowered LDL levels (P=0.006) but was unable to increase HDL levels (P=0.190). The *in vivo* tests showed that the ethyl acetate fraction of *I. cylindrica* reduced total cholesterol and LDL levels more effectively than the ethanol extract, but did not affect HDL levels in rats with hypercholesterolemia.

Correspondence to: Dr Siti Khaerunnisa or Professor Soetjipto Soetjipto, Department of Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Campus A Universitas Airlangga, 47 Prof Dr. Moestopo, Surabaya, East Java 60132, Indonesia
E-mail: st.khaerunnisa@fk.unair.ac.id
E-mail: soetjipto@fk.unair.ac.id

Abbreviations: Apo-A1, apolipoprotein-A1; BW, body weight; CD, control diet; CE, cholesterol ester; *I. cylindrica*, *Imperata cylindrica*; CVD, cardiovascular disease; GAE, gallic acid equivalent; HD, hypercholesterol diet; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NMR, nuclear magnetic resonance; ^{1}H-NMR, proton nuclear magnetic resonance; ^{13}C-NMR, carbon nuclear magnetic resonance; Rf, retention factor; TLC, thin-layer chromatography

Key words: Imperata cylindrica, flavonoid, 7,3',5'-trimethoxyflavonol, ethanol, ethyl acetate, hypocholesterolemic agents

Introduction

Cogon grass (*Imperata cylindrica*/*I. cylindrica*) is a member of the Gramineae family and is ubiquitously found in nature, but is often considered useless and treated as a weed (1). However, this plant is widely utilized within traditional medicine, particularly in Asia (2). Various studies have shown that *I. cylindrica* exhibits numerous beneficial biological properties, as it contains glycoside, triterpenoids, flavonoids, phenolic compounds, tannins and proteins, which display antioxidant properties *in vitro* (3-5). Cogon grass reeds are also suitable for use as an alternative medication to treat hypercholesterolemia (6). Patients with hypercholesterolaemia are at increased risk of developing cardiovascular diseases or suffering from a stroke, which can result in death at a young age, adding to the global disease burden (7).

Various studies have shown that hypercholesterolemia and cardiovascular disease are associated with the overproduction of reactive oxygen species (8-10). These free radicals facilitate lipid peroxidation within cell membranes, which results in the production of radical lipid peroxides and various other free radicals (11). Free radicals possess unpaired electrons, which are unstable and exhibit a tendency to absorb electrons from other molecules within close proximity to them in order to achieve stability (12,13). This can cause damage to various components of the cell (14).

Phenolic compounds located within *I. cylindrica* roots, such as flavonoids, oligostilbenoid and phenolic acids, are compounds that display strong antioxidant activity (15). Antioxidants serve an important role in the process of

scavenging free radicals and breaking down oxidation chain reactions both *in vitro* and *in vivo* (14). The antioxidant activity of phenolic compounds is induced primarily via the presence of hydroxyl groups (-OH) within their aromatic ring structures, mediating redox reactions in a specific way to neutralize free radicals (16).

In the present study, flavonoid compounds from *I. cylindrica* roots were isolated and purified through several stages, and *in vivo* tests were used to assess the effects of *I. cylindrica* on lipid profile levels in a rat model of hypercholesterolaemia.

Materials and methods

I. cylindrica samples. The plant material used for isolation and identification in the present study was *I. cylindrica* roots obtained from the Punjul village, Karangrejo District, Tulungagung Regency, and farmland in Malang, East Java, Indonesia. The plant material utilized for *in vivo* evaluation of lipid-lowering properties was identified and confirmed by the Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Indonesia.

Isolation and identification of I. cylindrica root compounds. A total of 850 g *I. cylindrica* roots powder was extracted by maceration using 96% methanol as a solvent three times, 24 h each, at room temperature. Subsequently, the filtrate and the residue were separated by filtering through a Whatman 42 filter paper (Whatman, plc; GE Healthcare) with a pore size of 2.5 μm. The methanol extract was then evaporated with a rotary vacuum evaporator (BÜCHI® R210; BÜCHI Labortechnik, AG) to yield a thick extract.

The thick methanol extract was then added to water and partitioned with *n*-hexane (1:1), followed by partitioning with ethyl acetate (1:1). The separation was performed using thin-layer chromatography (TLC). The ethyl acetate fraction was screened for flavonoids using Willstatter reagents, and the target flavonoid compound to be isolated was found in this fraction.

A total of 14 g ethyl acetate viscous extract obtained was absorbed in 28 g silica gel G 60 of size 0.2-0.5 mm. The compounds were then separated using gravity column chromatography. The eluent used was *n*-hexane ethyl acetate, starting with a ratio of 9:1 with an increasing polarity gradient, followed by 100% ethyl acetate and 100% methanol. From this separation process, 24 fractions were obtained, which were then grouped into 6 main fractions based on similar retention factor (Rf) values in a TLC test using *n*-hexane and ethyl acetate (2:8) eluate, termed fractions A, B, C, D, E and F.

The combined fractions of B and C were then targeted for this study and were separated and purified further. The combined fractions were absorbed in silica gel as above. Then, the compounds were separated by gravity column chromatography using a hexane-chloroform mixture eluent, starting with a ratio of 7:3, with an increasing polarity gradient, followed by 100% chloroform, 100% ethyl acetate and 100% methanol. Thus, 121 fractions were obtained, which were then grouped into five main fractions based on similar Rf values in a TLC test using 100% chloroform eluent, termed fractions G, H, I, J and K.

The combined fractions of I and J were then absorbed in silica gel. The compounds were separated by gravity column chromatography using the chloroform-*n*-hexane eluent, starting with a ratio of 19:1 with an increasing polarity gradient, followed by 100% chloroform and chloroform-ethanol eluent starting at 19:1, with an increasing polarity gradient to a ratio of 19:1, followed by 100% ethyl acetate and 100% methanol. From the elution process, 64 fractions were obtained, which were subsequently grouped based on similar Rf values in a TLC test using chloroform-ethanol (97:3) eluent into four main fractions, termed fractions L, M, N and O, in which the target compound was in the M and N fractions.

The M and N fractions were combined and further purified by gravity column chromatography using *n*-hexane ethyl acetate (9:1) as the eluent to obtain a pure yellow compound with a yield of 5 mg. The pure compounds obtained after showing a single spot on TLC were then identified using proton core nuclear magnetic resonance spectroscopy (^{1}H-NMR) and carbon core nuclear magnetic resonance spectroscopy (^{13}C-NMR). NMR spectroscopic analysis was performed using NMR JEOL ALPHA 500, 1H 500 MHz and 13C 125 MHz, at the Faculty of Pharmacy, Meijo University, Nagoya, Japan.

Determination of total phenolic content. The total phenolic content was determined using the Folin-Ciocalteu method (17,18). The ethanol extract/ethyl acetate fraction of *I. cylindrica* was oxidized using Folin-Ciocalteu reagent (Merck KGaA) and the reaction was neutralized using 20% Na_2CO_3. Absorbance was measured by exciting with blue light at a wavelength of 760 nm after 60 min, employing standard gallic acid. Total phenolic content is presented in units of g gallic acid equivalent (GAE) per kg of *I. cylindrica* extract/fraction. Measurements were taken three times, and the mean was taken (19).

Assessment of in vivo lipid-lowering properties. The present study was approved by the Animal Care and Use Committee, Faculty of Medicine Universitas Airlangga (Surabaya, Indonesia). All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (20). A total of 28 male Wistar rats aged 2-3 months, weighing 180-200 g in a healthy condition were used in the present study. The animals were acclimatized to the laboratory conditions for 7 days, provided *ad libitum* access to food and water, and maintained under a 12 h light/dark cycle at room temperature. After acclimatization, the animals were divided randomly into four groups. The present study utilized a randomized post-test only control group experimental design. Group 1 (K0): A negative control group that received a control diet (CD); group 2 (K1): A positive control group that received a high cholesterol diet (HD); group 3 (K2): A treatment group that was administered a HD and the ethanol extract of *I. cylindrica* at a dose of 15 mg/200 g body weight (BW); and group 4 (K3): A treatment group that was administered a HD and the ethyl acetate *I. cylindrica* fraction at a dose of 15 mg/200 g BW [based on Suratman *et al* (21) with modifications].

Rats were used in the present study as they have similar physiological and behavioural characteristics as humans (22). The anatomical structure of rats is slightly different from those of other mammals, for examples, the oesophagus directly empties into the stomach such that the rat cannot spit out its food

and they possess no gallbladder ties. At the age of 3 months, these rats are mature and their anatomy and physiology are optimal; the male rat does not possess an oestrus cycle, so it does not affect blood cholesterol levels (23,24).

For the *in vivo* test, re-extracts were created by replacing the methanol with ethanol in the aforementioned extraction procedures to obtain the ethyl acetate fraction. Ethanol was used due to its very low toxicity. The present study employed a single dose of 15 mg/200 g BW, according to the method described by Suratman *et al* (21) with slight modifications. Ethanol extracts were composed of various compounds, while the ethyl acetate fraction was a fraction comprised of semipolar compounds. The mean weight ± standard deviation of the rats was 197.28±10.78 g, thus, each rat received 15 mg of the extract, which was dissolved in 2 ml carboxymethyl cellulose sodium (1 ml/100 g BW).

Hypercholesterolemia was determined on the 15th day following induction with a HD, which was considered sufficient based on the increase in total blood cholesterol levels >54 mg/dl, with a normal value of 10-54 mg/dl (23,24).

Preliminary tests demonstrated that the administration of HD for 14 days resulted in increased blood cholesterol levels, and hypercholesterolemia persisted via continuation of a HD until the 30th day (data not shown). Thus, on days 15-30, the treatment group was administered 15 mg/200 g BW (K2) ethanol extract and 15 mg/200 g BW (K3) ethyl acetate fraction. The administration of HD within the control group (K1) and the treatment group (K2 and K3) was continued until the 30th day.

On the day of the sacrifice (end of 5th week/day 36), the rats were anesthetized by a 0.1 ml/100 g BW intraperitoneal injection of a cocktail of drugs (ketamine 50 mg/kg, xylazine 2 mg/kg and acepromazine 0.5 mg/kg) (25). All rats were sacrificed by collecting the blood from the cardiac vein, and following drawing of blood, the arteries were cut to ensure termination of the animals.

HD was composed of 12.5% casein, 20% pork oil, 1% cholesterol, 0.25% cholic acid, 20,52% sucrose, 41,23% flour, 3,5% salt mixture and 1% multivitamins [based on and modified from Mohamed *et al* (26)]. The administration of HD lasted for one month. The CD was composed of 12,5% casein, 10% corn oil, 23,3% sucrose, 46,7% flour, 3,5% salt mixture, 1% multivitamins and 3% fibre (26). The feed was formed into pellets and was provided *ad libitum* every day for 30 days. Determination of total cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol levels was outsourced to Surabaya Regional Health Laboratory were enzymatic methods and spectrophotometry were used.

Statistical analysis. Data were analysed using SPSS version 23 (IBM, Corp.). Comparisons between total cholesterol, HDL and LDL levels were assessed using ANOVA, followed by a least significant difference test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Identification of isolated compounds. The signals in ^{13}C-NMR [insolvent $(CD3)_2$ CO] presented at chemical shift (δ) in parts per million (ppm) values of 206.11, 167.5, 152.07, 148.07, 124.85, 122.89, 115.53, 113.46 and 56.3. The signal at δ 206.11 ppm was the C-carbonyl signal, whereas the signals at δ 167.5, 152.07, 148.07, 124.85, 122.89, 115.53 and 113.46 ppm represented 14 carbon atoms in a compound comprised 12 carbon atoms from the two aromatic rings on rings A and B (which were subdivided into five C-aryloxy atoms and 7 aromatic carbon atoms) and two ethylenic carbon signals on ring C. A shift of δ 56.3 ppm indicated the presence of a methoxy carbon signal (OCH_3).

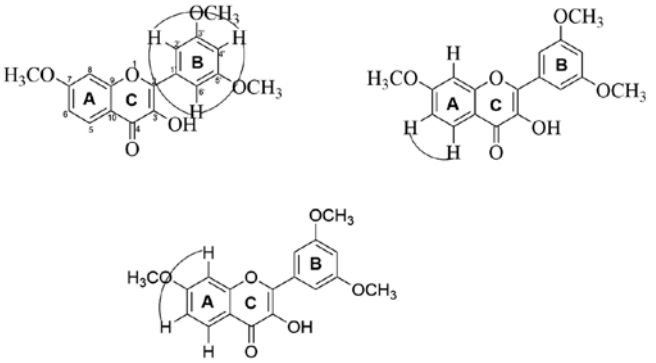

Figure 1. Protons arranged at coupling positions of the isolated flavonoid compound. A, B and D show the position of the rings of the flavonoid.

^{1}H-NMR proton spectroscopy analysis in the $CDCl_3$ solvent revealed that the isolated flavonoid has 6 aromatic protons at δ ppm 7.70 [1H, doublet (d), J=1.5 Hz], 7.68 (1H, d, J=2.2 Hz), 7.56 (2H, d, J=2.2 Hz) and 6.94 (2H, d, J=8.4 Hz). The 6-proton-signal aromatic was a doublet distributed over positions, such that for the meta position, the signal was δ 7.68 ppm (1H, d, J=2.2 Hz) and δ 7.56 ppm (2H, d, J=2.2 Hz), whereas the proton doublet signal δ 7.70 ppm (1H, d, J=1.5 Hz) represented a mutual position at an ortho position to the proton signal δ 6.94 ppm (2H, d, J=8.4 Hz). Signals at δ ppm 7.56 and 6.94 each represented two protons (visible from the signal intensity, which was two times higher than signals at δ ppm 7.70 and 7.68). A singlet signal at d ppm 3.87 (9H, s) indicated the presence of three OCH_3 substituents.

The results of ^{1}H-NMR analysis were as follows: d ppm 7.68 (1H, d, J=2.2 Hz), d ppm 7.56 (2H, d, J=2.2 Hz), d ppm 7.70 (1H, d, J=1.5 Hz) and d ppm 6.94 (2H, d, J=8.4 Hz) (Fig. 1).

Tables I and II display the NMR spectral analysis of isolated compounds in comparison to previous studies covering a structure similar to the isolated compounds (27,28). From the analysis, the recommended structure for the compound as a result of isolation is shown in Fig. 2, noting the chemical shift data for each H and C atom.

Total phenolic content. Total phenolic content is expressed as g GAE/kg extract by comparing the standard gallic acid curve with the absorbance curve of the sample. Both ethanol extracts and ethyl acetate fractions were screened using Willstater reagent, and they produced an orange colour, indicating a positive result for the presence of a flavonoid compound. The determination of total phenolic content was performed using the Folin-Ciocalteau method. In the ethanol extract *I. cylindrica*, the total phenolic content was 545.67 g GAE/kg

Table I. Comparison of δ ^{1}H-NMR for isolated flavonol compounds based on previous studies.

H position	d Isolated compound	d Quercetin (27)	d Kaempferol (27)
H_1	-	-	
H_2	-	-	-
H_3	-	-	-
H_4	-	-	-
H_5	6.94 ppm, d, *J*=8.4 Hz	-	-
H_6	6.94 ppm, d, *J*=8.4 Hz	6.37 ppm, d, *J*=2.5 Hz	6.2 ppm, d, *J*=8 Hz
H_7	-	-	-
H_8	7.70 ppm, d, *J*=1.5 Hz	6.14 ppm, d, *J*=2.5 Hz	6.4 ppm, d, *J*=8 Hz
H_9	-	-	-
H_{10}	-	-	-
H_1'	-	-	-
H_2'	7.56 ppm, d, *J*=2.2 Hz	7.64 ppm, d, *J*=8.5 Hz	8.0 ppm, d, *J*=8 Hz
H_3'	-	-	6.9 ppm, d, *J*=8 Hz
H_4'	7.56 ppm, d, *J*=2.2 Hz	-	-
H_5'	-	6.85 ppm, d, *J*=8.5 Hz	6.9 ppm, d, *J*=8 Hz
H_6'	7.68 ppm, d, *J*=0.2 Hz	7.49 ppm, q, *J*=8.5 Hz	(8.0 ppm, d, *J*=8 Hz
H-OMe	3.87 ppm, s	-	-
OH	-	-	-

H, proton; OH, hydroxyl; ppm, parts per million; s, singlet; d, doublet; t, triplet; q, quartet; δ, chemical shift.

Table II. Comparison of δ ^{13}C-NMR for isolated flavonol compounds based on previous studies.

C position	Isolated compound δ, ppm	Quercetin δ, ppm (27)	Quercetin δ, ppm (28)
C_2	124.8	156.6	146.8
C_3	124.8	136.2	135.5
C_4	206.11	176.3	175.8
C_5	115.53	161.2	160.7
C_6	115.53	98.8	98.2
C_7	152.07	164.5	163.9
C_8	113.46	93.7	93.3
C_9	148.7	148.2	156.2
C_{10}	122.8	103.4	103.1
C_1'	124.8	120.4	122.1
C_2'	113.46	116.1	115.3
C_3'	167.5	145.5	145.0
C_4'	113.46	147.2	147.6
C_5'	167.5	115.5	115.6
C_6'	113.46	122.4	120.0

C, carbon; ppm, parts per million; δ, chemical shift.

extract. In the ethyl acetate fraction of *I. cylindrica*, total phenolic content was 682.33 g GAE/kg extract.

In vivo lipid-lowering properties. The effect of extracts were assessed *in vivo* for 5 weeks. At the end of the 5th week/day 36, the animals were sacrificed and ~5 ml blood was collected from the cardiac vein.

At the end of the treatment (end of 5th week/day 36), animals from each group (K0, K1, K2 and K3) were weighed. Total cholesterol, HDL and LDL levels were assessed in the blood samples. The results showed that the hypercholesterolemia diet in the experimental group increased cholesterol levels significantly (Table III and Fig. 3).

Effect of ethanol extract and ethyl acetate fraction on total cholesterol levels. Treatment with the ethanol extract (15 mg/200 g BW; K2) lowered the total cholesterol levels significantly (P=0.001; mean ± standard deviation, 60.43±7.6 mg/dl) compared with the positive control group (K1). Treatment with the ethyl acetate fraction (15 mg/200 g BW; K3) also lowered the total cholesterol levels significantly (P=0.002; mean ± standard deviation, 70.57±9.2 mg/dl) compared with the positive control group (K1) (Table III and Fig. 3).

Effect of ethanol extract and ethyl acetate fraction on LDL levels. Treatment with the ethanol extract (K2) reduce LDL levels but the difference was not significant (P=0.109; mean ± standard deviation, 11.57±2.3 mg/dl) compared with the positive control group (K1). The ethyl acetate fraction (K3) significantly reduced LDL levels (P=0.006; mean ± standard deviation, 9.86±2.2 mg/dl) compared with the positive control group (K1) (Table III and Fig. 3).

Effect of ethanol extract and ethyl acetate fraction on HDL levels. The ethanol extract (K2) significantly reduced HDL levels (P=0.003; mean ± standard deviation, 21.14±2.8 mg/dl compared with the positive control group (K1). The ethyl acetate

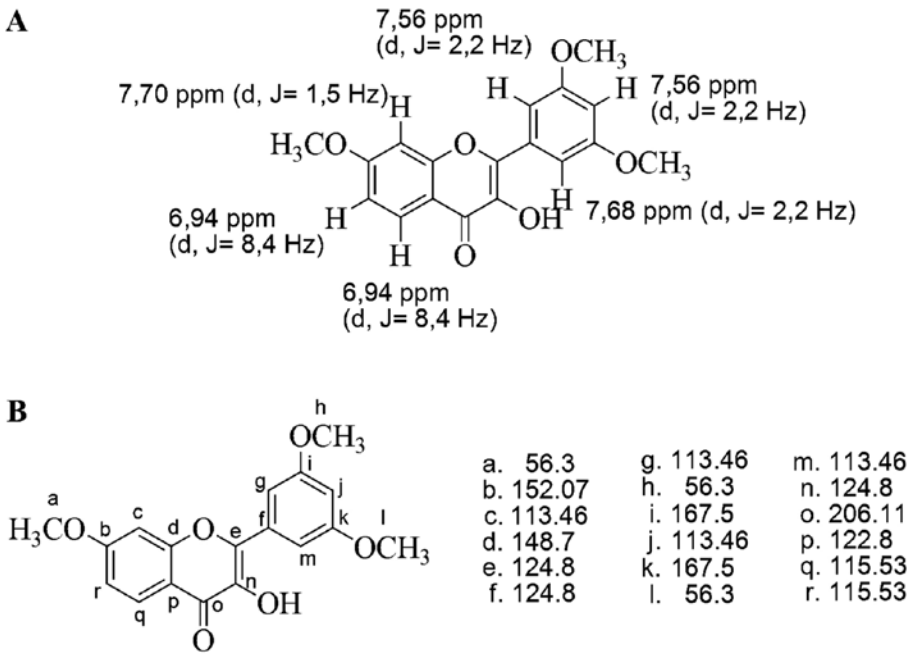

Figure 2. Actualization of (A) proton and (B) carbon positions of the isolated flavonoid compound. ppm, parts per million; Hz, Hertz; d, duplet.

fraction (K3) did not significantly reduce HDL levels (P=0.190; mean ± standard deviation, 25±4.1 mg/dl compared with positive the control group (K1) (Table III and Fig. 3).

Discussion

Based on the results of the analysis of ^{1}H-NMR and ^{13}C-NMR, the isolated compound was considered to be a flavonoid of the flavonol group, and consisted of 18 carbon atoms and 16 hydrogen atoms, termed 7,3',5'-trimethoxyflavonol. The ethyl acetate fraction of *I. cylindrica* extraction was rich in flavonoid compounds.

A comparison of total serum cholesterol levels between the CD (K0) and HD-fed (K1) groups showed that the HD induced hypercholesterolemia *in vivo*. The present study demonstrated that a diet of pork fat and pure cholesterol for 4 weeks was successful in raising rat blood cholesterol levels in the K1 group.

The results demonstrated that the administration of the ethanol extract (K2) or the ethyl acetate fraction (K3) of *I. cylindrica* was capable of significantly reducing total cholesterol levels compared with the HD group (K1). The administration of the ethyl acetate fraction of *I. cylindrica* also significantly reduced LDL levels, and in groups treated with the ethanol extract, *I. cylindrica* extract did not significantly reduce cholesterol levels compared with the HD group. The administration of the ethyl acetate fraction of *I. cylindrica* did not exert a significant effect on HDL levels (P>0.05), and in the group administered with the ethanol extract of *I. cylindrica*, HDL levels were significantly reduced compared with the group fed an HD.

Based on previous studies, screening of the ethanol extracts of *I. cylindrica* showed the presence of tannins, saponins, flavonoids, alkaloids, cardiac glycosides, coumarin and terpenoids (29,30). It has also been demonstrated that *I. cylindrica* is a good source of antioxidants, including a high content of flavonoid and total phenolic compounds (4). Zhou *et al* (31) also showed that Rhizomes *Imperata* extract is rich in polyphenols, which also exhibit antioxidant activity.

The decrease in total cholesterol levels in the group administered ethanol extract was larger compared with the group administered the ethyl acetate fraction. Compounds contained within the ethanol extracts include flavonoids and saponins (32). Saponin binds with cholesterol and bile to form strong complex compounds and cannot be reabsorbed (33,34). This results in cholesterol elimination and increased bile excretion. The body attempts to increase the conversion of cholesterol into bile acids, thus reducing blood cholesterol levels (35).

The LDL cholesterol levels in the group administered the ethyl acetate fraction exhibited a larger decrease compared with the group administered the ethanol extract. In the ethyl acetate fraction, there were only semipolar compounds such as flavonoids, which reduce blood cholesterol levels by inhibiting cholesterol synthesis and increasing LDL receptor expression (36,37). Several studies have shown that the consumption of isoflavones induces a decrease in plasma cholesterol in C57BL/6 mice, increasing the activity of LDL receptors on HepG2 cells (37,38). This is likely due to the effect of flavonoids on sterol regulatory element-binding protein 2 (37). The ethyl acetate fraction consists of only semipolar compounds, and this is hypothesized to have a direct effect on LDL reduction, while the ethanol extract contains several complex compounds which exhibit antagonistic interactions between each other. Therefore, the effects of active constituents may be masked by other compounds in this complex mixture, thus making the LDL reduction effect of ethyl acetate fraction more potent than that of the ethanol extract. This phenomenon also occurs in mixtures of natural product (39). Semipolar compounds are considered to be more effective in regulating LDL uptake. The effect of reducing LDL cholesterol using the ethyl acetate fraction was better compared with the ethanol extract. It is hypothesized that the semipolar compounds increase LDL uptake, which can affect the regulation of LDL.

Table III. Total cholesterol, LDL and HDL levels *in vivo*.

Variables	K0[a]	K1[a]	K2[a]	K3[a]
Total cholesterol	74.29±4.3[b,e]	84.14±6.6	60.43±7.6[c]	70.57±9.2[c,d]
LDL	12.14±2.1	13.71±2.9	11.57±2.3	9.86±2.2[c]
HDL	26.29±3.1[d]	27.57±4.1	21.14±2.8[c]	25±4.1

[a]Mean ± standard deviation. [b]P<0.05, [c]P<0.01 vs. K1; [d]P<0.05, [e]P<0.01 vs. K2. LDL, low density protein; HDL, high-density protein; HD, hypercholesterol diet; BW, body weight; HD, high cholesterol diet; *I. cylindrica*, *Imperata cylindrica*; K0, negative control group fed a control diet; K1, positive control fed HD; K2, treatment group fed a HD and the ethanol extract of *I. cylindrica* at a dose of 15 mg/200 g BW; K3, treatment group administered a HD and the ethyl acetate *I. cylindrica* fraction at a dose of 15 mg/200 g BW; SD, standard deviation.

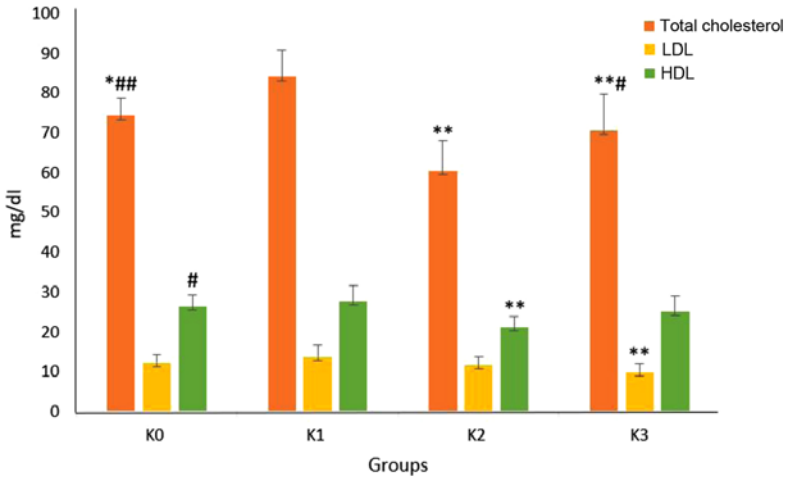

Figure 3. Total cholesterol, LDL and HDL levels in the experimental animal model. Data are presented as the mean ± standard deviation. N=6 per group. *P<0.05, **P<0.01 vs. K1; #P<0.05, ##P<0.01 vs. K2. LDL, low density protein; HDL, high-density protein; CD, control diet; HD, hypercholesterol diet; BW, body weight; HD, high cholesterol diet; *I. cylindrica*, *Imperata cylindrica*; K0, negative control group fed a control diet; K1, positive control fed HD; K2, treatment group fed a HD and the ethanol extract of *I. cylindrica* at a dose of 15 mg/200 g BW; K3, treatment group administered a HD and the ethyl acetate *I. cylindrica* fraction at a dose of 15 mg/200 g BW.

Flavonoids are reported in the literature to regulate apolipoprotein-B (apoB) secretion and cellular cholesterol homeostasis in human hepatoma cell lines, reducing ester cholesterol mass, inhibiting acyl-CoA: Cholesterol acyltransferase expression, inhibiting microsomal triglyceride transfer protein activity, and furthermore, inhibiting hepatic lipid synthesis by inhibiting apoB and increasing apoA levels in HepG2 cells (40).

Across all groups, the increase or decrease in HDL cholesterol levels could not be exclusively attributed to the administration of HD or the extract as it was suspected that the conditions of experimental animals were still in a compensated state; the positive control group (K1) showed high levels of HDL compared with other groups (K0, K2 and K3).This finding was supported by Dauqan *et al* (41), where, the normal stressed group of mice exhibited an increase in HDL levels, and Hayek *et al* (42) who suggested that a diet of saturated fat and a cholesterol diet facilitated an increase in HDL and Apo-A1 cholesterol levels.

Hayek *et al* (42) also suggested that a high-fat diet increased HDL levels, facilitated by an adaptation mechanism that is reflected in the increase in the flux of HDL, cholesterol ester (CE) and transport rate necessary when a high metabolic burden is created following a high-fat or high-cholesterol diet. A high-fat diet may reverse the transport of cholesterol via the HDL pathway. The administration of a high-fat diet can increase apolipoprotein-A1 (Apo-A1) levels via a posttranscriptional mechanism by increasing the translational ability of Apo-A1 mRNA and decreasing intracellular Apo-A1 degradation. High-fat diets can reduce the rate of catabolism of the HDL-CE and Apo-A1 fractions, which may be caused by an increase in HDL size. The duration of the diet is also considered a factor that influences the increase in HDL levels within the group fed a high-fat diet (42,43). This is further supported by Hayek *et al* (42), where administration of high-fat diets provided for 4 weeks resulted in an increase in HDL levels.

In general, the ethyl acetate fraction resulted in the most favourable effects, as the lipid-lowering profile included reduced total cholesterol and LDL levels, compared with the ethanol extract. This result is supported by the total phenolic contents of the two extracts, as the total phenolic content was higher in the ethyl acetate fraction compared with the ethanol extract.

The results of the present study are supported by previous studies that have shown the presence of an association between the intake of flavonoids such as flavones and flavonols and a decreased risk of coronary artery disease; even data on the intake of anthocyanins and flavanones showed

reduced mortality rates from coronary cardiovascular diseases (CVDs) (44-46). A meta-analysis demonstrated that the consumption of three cups of tea per day reduced the risk of CVD by 11%, while the consumption of red wine reduced the risk of CVD by 32% (47). A high intake of flavonoids from herbs was associated with a reduced risk of developing cardiovascular disease. The mechanism underlying their beneficial effects however, remain unclear, but current evidence suggests that flavonoids can affect cardiovascular risk factors (35).

A limitation of the present study was the use of a single dose for assessing the effects of the extracts and the fraction. Thus, the dose-dependency and optimal dose were not determined. Furthermore, quantitative tests of various compounds in the extracts and fractions were not performed; thus, synergy calculations could not be performed.

In summary, a pure compound was extracted from *I. cylindrica* roots in the form of a yellow powder. Based on the results of the analysis by ^{1}H-NMR and ^{13}C-NMR, the isolated compound was shown to be a flavonoid, which consisted of 18 carbon atoms and 16 hydrogen atoms and was termed 7,3',5'-trimethoxyflavonol. The *in vivo* tests showed that the ethyl acetate fraction of *I. cylindrica* reduced total cholesterol and LDL level more effectively than the ethanol extract but did not affect HDL levels in a rat model of hypercholesterolemia.

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Availability of data and materials

The datasets used and/or analysed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

SKh conceived and designed the study. NSA, ANK, SKh, SKu and SSu performed the sample collection. SKh, CDKW and SSo performed the laboratory experiments. SKh and CDKW analysed the data and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Faculty of Medicine, University of Airlangga (Surabaya, Indonesia) (approval no. 027/EC/KEPK/FKUA/2012).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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