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RESEARCH ARTICLE

The effect of ethanol extract from *Portulaca oleracea* on inhibiting total cholesterol on animal subjects

Afifah Bambang Sutjiatmo, Fahmy Ahsanul Haq, Sulaeman Al Jati, Suci Nar Vikasari

Faculty of Pharmacy, Universitas Jenderal Achmad Yani, Cimahi, West Java, Indonesia

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Correspondence
Afifah Bambang Sutjiatmo
Faculty of Pharmacy
Universitas Jenderal Achmad Yani
Cimahi
West Java
Indonesia
afifah@lecture.unjani.ac.id

Abstract

Introduction: Hypercholesterolemia occurs when cholesterol levels in the blood increases. Traditionally, *krokot* (*purslane*, *portulaca oleracea*) is used to treat cardiovascular disease. **Aim**: This research evaluated the effect of purslane extract to inhibit increasing of cholesterol levels. **Methods**: The ethanol extract dosage of purslane was 27.5, 55, and 110 mg/kg body weight (bw) and simvastatin 1.8 mg/kg bw were used as comparisons. The anti-hyper cholesterol effect test was done by feeding a high cholesterol diet and drinks containing 0.01% propylthiouracil. The test parameters were body weight and total cholesterol levels on days 0, 7, 14, and 21. **Results**: The results showed that the extract was able to prevent the increase in body weight compared to the control group (p<0.05) and that it could inhibit the increase of total cholesterol levels at day 14 and 21 compared to control group (p<0.05) and equivalent to simvastatin (p>0.05).

Introduction

Modernisation always changes lifestyles and habits, including excessive eating, doing too many activities, and too much smoking, but lack of resting. These changes can result in diseases including coronary heart disease and atherosclerosis, which occur when cholesterol levels in the blood increase. If blood cholesterol levels increase, then drugs which are called antihyperlipidemic (hypolipidemic), are needed to decrease the levels (Steyn & Damasceno, 1991). At present, available medicines are relatively expensive, making them unaffordable, especially for low-income people. Therefore, people began to turn to traditional medicine or known as going back to nature. Some plants have traditionally been used to treat hyper cholesterol disease, including *ceremai*, starfruit, purslane, and *binahong*. Purslane (*Portulaca oleraceae*), known as *krokot* in Indonesia, besides being used to feed animals, also has benefits for humans. Purslane can be used as an antioxidant, anti-diarrhoea, haemorrhoids medicine, anti-arthritic, anti-diabetic, hepatoprotective, nephroprotective, anti-inflammatory, anti-hypertension, and to help blood circulation (Jacob *et al*., 2017). The chemical content of purslane includes omega 3 fatty acid, alpha-linolenic acid, and eicosapentaenoic acid. Purslane herbs also contain vitamin A, vitamin C, vitamin B, and carotenoids. Other phytochemicals are alkaloids, terpenoids, organic acids, coumarins, flavonoids, essential oils, and polysaccharides (Uddin *et al*., 2014; Jacob *et al*., 2017; Petropoulos *et al*., 2019). The purslane-leaf extract showed a hypolipidemic effect in rats that were fed with high cholesterol diet (Shanker & Debnath, 2016). The effect of 70% ethanol extract from purslane leaves in rabbits fed with high cholesterol showed that the extract at doses of 200, 400, and 800 mg/kg bw per oral could decrease low density lipoprotein significantly compared to the control group (Movahedian *et al*., 2007). When 96% ethanol extract from purslane herbs at doses of 200, 400 and 800 mg/kg bw were given via intraperitoneal route, they also decreased the cholesterol and triglycerides levels even though the plasma concentration remained high and showed no

significant difference compared to the control group (Ashtiyani, 2016). Purslane stem extract also showed hypolipidemic effect on Wistar rats fed with hyperlipidemic diet that contained 20% fat, 1% cholesterol and 0.25% colic acid (El-Newary, 2016). Therefore, this research examined the effect of 50% ethanol extract from purslane herbs on hyper cholesterol rats.

Material and method

Collection of purslane herbs

The purslane herbs (Figure 1) used in this research were collected from Manoko plantations in Lembang, West Java, Indonesia. Determination of the purslane herbs was done at the Faculty of Biology, Padjadjaran University, with authentic identification No. 458/HB/01/2017.

Figure 1: ***Portulaca oleracea***

Extraction of purslane herbs

The extraction of the purslane herbs was done with 50% ethanol using continuous extraction (Soxhlet), and the thickening was done using a vacuum evaporator. The thickened extract was dried at ±60°C. The dried extract was pollinated with grinders, sifted, and stored in containers in drying cupboards at ± 60°C or in drying cupboards with a damp absorber (desiccant).

Examination of characteristics and phytochemical screening of Simplicia and purslane extract

Examination of the characteristics of Simplicia and the extract includes determining the water-soluble extract content, determining the ethanol-soluble content, determining the drying losses, determining the water content, and determining the total ash content. Phytochemical screening of Simplicia and the extract include examination of flavonoids, saponins, alkaloids, polyphenols, tannins, steroids and triterpenoids, quinones, monoterpenoids, and sesquiterpenoids (World Health Organization, 2011).

Antihipercholesterol effects assay

The experiment was conducted according to the procedure approved by the institutional ethic committee No. 6022/KEP-UNJANI/VII/2019. The experimental animals used were male Wistar rats obtained from the Animal Laboratory of Bioscience and Biotechnology Research Center of Institut Teknologi Bandung. The animals were divided into five groups, each consisting of four rats, namely the control group (CMC 0.5%), reference group (Simvastatin at a dose of 0.9 mg/kg bw), and purslane ethanol extract groups at doses of 27.5, 55, and 110 mg/kg bw. At the beginning of the study, the total cholesterol levels were determined. The test animals were induced exogenously and endogenously. Exogenous induction was done by giving high cholesterol foods. Endogenous induction was carried out by giving drinks containing propylthiouracil (PTU) 0.01% *ad libitum*. The composition of high cholesterol food was 1% pure cholesterol (Sigma-Aldrich, Cat. No. C8503), 20% goat fat, 5% duck egg yolk, 10% cooking oil, 10% beef liver, and standard feed ad 100%. The experiment was carried for 21 days, and the extract was given orally on a daily basis. Blood sampling and determining the cholesterol levels were carried out on days 7, 14, and 21. Blood sampling was done through the tail vein. Measurement of total blood cholesterol levels was carried out using an enzymatic reaction (reagent kit BioMaxima, Cat. No. 1-023-1000) using a Spectrophotometer (Clinicon 4010) at 546 nm. The collected data were analysed using *t-test* statistics.

Results

Examination of characteristics and phytochemical screening

The aim of examining the characteristics of purslane herbs was to standardise the materials and to find out the general criteria for the quality of materials to be used. The results of the characterisation of purslane Simplisia and ethanol extract are presented in Tables I and II. The results showed that both of the purslane herbs, Simplicia and ethanol extract, contained alkaloid, polyphenol, tannins, flavonoid, quinones, terpenoid, and steroids. Saponin was only showed in purslane Simplisia.

Table I: The results of the examination of the characteristics of purslane herbs

Parameter	Result (%)
Total Ash Level (% w/w)	25.00 ± 1.43
Water Soluble Ash Level (% w/w)	15.18 ± 1.14
Unsolved Acid Level (% w/w)	15.82 ± 0.08
Water Content (% v/w)	8.00 ± 0.01
Water Soluble Content (% w/w)	36.18 ± 0.76
Ethanol Soluble Content (% w/w)	12.99 ± 0.40

Antihipercholesterol effects assay

In the assay of the hypolipidemic effect of ethanol extract of purslane herbs, the body weight and total blood cholesterol levels of the subjects were observed. Observation results of body weight are presented in Table III, while the results of measurements of total cholesterol levels are depicted in Figure 2.

Table II. Phytochemical screening of Simplicia and ethanol extract of purslane herbs

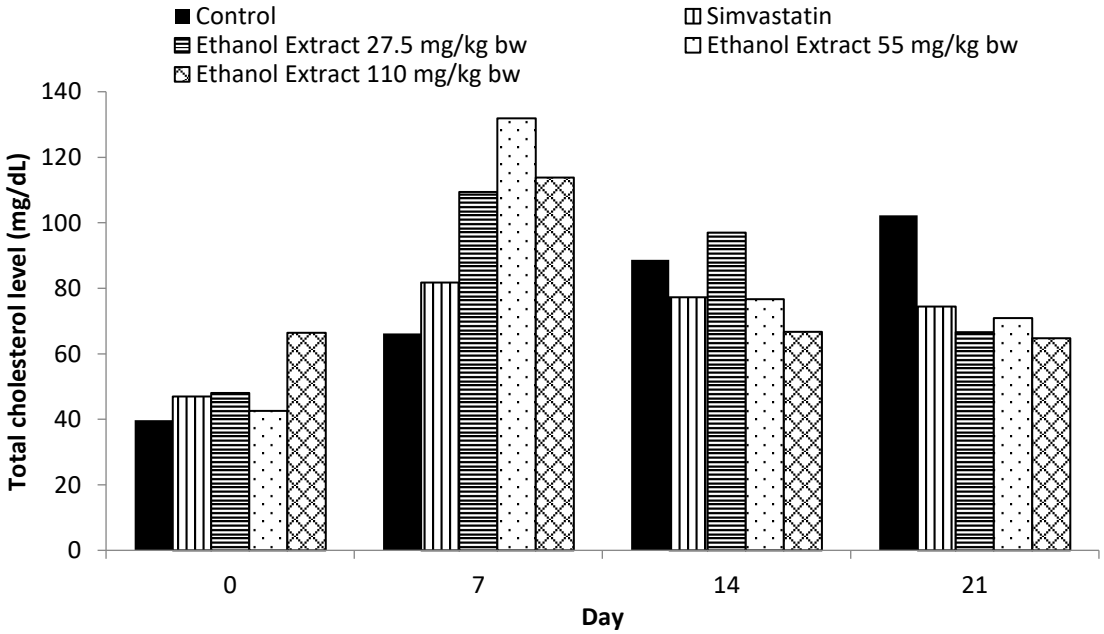
Compound	Result	
	Simplicia	Ethanol extract
Alkaloid	+	+
Polyphenol	+	+
Tannin	+	+
Flavonoid	+	+
Quinones	+	+
Saponin	+	-
Monoterpen and Sesquiterpen	+	+
Steroid and Triterpenoid	+	+

+: Positive contains test compounds
-: Negatives contain test compounds

Table III: Animal body weight on hypolipidemic assay

Group	Body weight (gram) at day			
	0	7	14	21
Control	255.3±47.8	262.5±53.2	290.0±50.3	298.0±50.6
Simvastatin	217.8±33.7	224.3±26.2*	241.5±25.7*	246.5±30.8
Ethanol extract 27.5 mg/kg bw	225.8±32.2	230.8±25.7*	244.0±20.2*	239.3±21.8*
Ethanol extract 55 mg/kg bw	215.3±21.3	221.0±26.0*	238.3±31.0*	210.8±36.4*
Ethanol extract 110 mg/kg bw	220.3±16.7	223.5±19.8*	240.8±18.6*	238.3±19.3*

n=4, *p<0.05 compared to control group using t-test



n=4, *p<0.05 compared to control group using t-test

Figure 2: Total cholesterol level on hypolipidemic assay

The observation of body weight showed that the ethanol extract was able to inhibit the increase in body weight from day 7 to day 21. Ethanol extract at a dose of 27.5 mg/kg bw was able to inhibit the increase in body weight by 10.77% on day 21. The administration of ethanol extract at a dose of 55 mg/kg bw was able to inhibit body weight gain by 18.84% on day 21. The administration of ethanol extract at a dose of 110 mg/kg bw was able to inhibit body weight gain by 8.58% on day 21. In contrast, the reference drug simvastatin was able to inhibit body weight increase by 3.55% on day 21. The optimal result of inhibition body weight gain was shown by ethanol extract at a dose of 55 mg/kg bw.

The measurement of total blood cholesterol level showed that the per cent relative level of total cholesterol in the serum of ethanol extract group dose 27.5 mg/kg bw on day 21 was 118.53% lower than the control group, while the total cholesterol level of the extract 55 mg/kg bw was 90.86% lower than the control group, and the total cholesterol level of the extract 110 mg/kg bw was 159.80% lower than the control group. This showed that the ethanol extract could apparently reduce the total cholesterol levels in the serum even though it was not different from the control group (p>0.05).

Discussion

In this research, the administration of purslane extract to inhibit the increase of total blood cholesterol level was carried out using exogenous and endogenous induction. The endogenous induction was carried out by giving cholesterol and PTU. The induction by giving pure cholesterol was done because it will contribute to the increase of cholesterol levels by as much as 70-80% on cholesterol levels in the liver, small intestines, and adrenal glands. The test animals were also given a high cholesterol diet with an increase of 10-30% (Dietschy & Siperstein, 1967). The induction was also done by giving PTU, which is a drug used to reduce thyroid levels. Clinical studies state that thyroid hormones will influence the formation of cholesterol, especially low-density cholesterol (LDL) (Abrams *et al.*, 1981). In testing using animals, this condition can increase body weight (Suzuki *et al*., 1979).

Purslane ethanol extract could prevent weight gain when given together with foods high in cholesterol. This study is in line with the research conducted by Hussein (2010), which stated that 95% ethanol extract of purslane obtained through extraction using Soxhlet also showed significant inhibition of body weight gain, blood glucose, triglyceride, total cholesterol, LDL-C, HDL-C, free fatty acids, and the atherogenic index levels in a dose-dependent manner on obesity-induced diabetic rats fed by a high-fat diet (Hussein, 2010). In contrast, research conducted by Shafi & Tabassum (2018) stated that 50% ethanol extract of purslane obtained through maceration could increase the bodyweight of animals in streptozotocin-induced diabetic (Shafi & Tabassum, 2018). This difference could be due to different extraction methods, in which this research used continuous heat extraction.

Different extraction methods will affect the compounds contained in the extract. Maceration is an extraction suitable for the isolation of thermolabile compounds, while the Soxhlet extraction at high temperatures and long extraction time can increase compound degradation due to temperature (Zhang *et al*., 2018).

The effects of the anti-cholesterol of purslane extract were likely to be the result of flavonoids. Flavonoids have antioxidant effects, and they can affect cholesterol concentration, especially LDL levels. Flavonoids will inhibit LDL oxidation, thereby reducing the likelihood of injury in the endothelial wall and reducing the risk of arteriosclerosis (Nijveldt *et al*., 2001).

One of the factors that influence the occurrence of hyperlipidemia is oxidative stress. Purslane contains high antioxidants, including alpha-tocopherol and ascorbic acid, so it is useful for reducing oxidative stress (Uddin *et al*., 2014). Vitamin A and carotenoids can prevent free radicals and LDL peroxidation. Beta carotene can lower blood cholesterol levels by preventing the HMGCoA reductase enzyme activity. Carotenoids also increase macrophage LDL receptor activity and reduce circulating LDL, inflammation, oxidative stress, and endothelial dysfunction (Malekmohammad *et al*., 2019). Purslane also contains omega 3 fatty acids, which are useful for increasing high-density lipoproteins and reducing blood viscosity.

It can be concluded that 50% ethanol extract of purslane herbs has the potential to inhibit the increase of total blood cholesterol levels in an animal model.

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