Pharmacy Education (2021) 21(2) 184 - 188
https://doi.org/10.46542/pe.2021.212.184188

IAI CONFERENCE

RESEARCH ARTICLE

In vivo activity of *Phaseolus vulgaris* as an anti-hypercholesterolemic

Keni Idacahyati, Yedy Purwandi Sukmawan, Nopi Yanti, Winda Trisna Wulandari, Firman Gustaman, Indra

STIKes Bakti Tunas Husada Tasikmalaya, Tasikmalaya, Indonesia

Keywords
Antihyperlipidemic
Cholesterol
Extraction
Green beans
Phaseolus vulgaris

Correspondence
Keni Idacahyati
Department of Pharmacology and Clinical Pharmacy
STIKes Bakti Tunas Husada Tasikmalaya
Cilolohan Street No. 36
Tasikmalaya
Indonesia
keniida@stikes-bth.ac.id

Abstract

Introduction: Hyperlipidemia occurs due to increased levels of lipids and cholesterol in the blood. Phytosterols, such as stigmasterol in *Phaseolus vulgaris*, can reduce blood cholesterol levels. **Aims**: The purpose of this study was to determine the activity of *P. vulgaris* in hypercholesterolemic rats. **Methods:** Nine groups underwent the anti-hypercholesterolemia test: control group, negative group, positive group, 150 mg/kg body weight (bw) and 300 mg/kg bw n-hexane extract groups, 150 mg/kg bw and 300 mg/kg bw ethyl acetate extract groups, and 150 mg/kg bw and 300 mg/kg bw ethanol extract groups. **Results:** All groups, except the control group, were given a high cholesterol diet to induce hypercholesterolemia (until the total cholesterol levels were higher than 200 mg/dL), followed by testing for ten days. The results showed that the 300 mg/kg bw ethyl acetate extract group had the best activity in reducing total cholesterol.

Introduction

Hyperlipidemia is a medical condition characterised by an increase in one or more of the plasma lipids (triglycerides, cholesterol, cholesterol esters, and phospholipids) and/or plasma lipoproteins (very-low-density lipoprotein and low-density lipoprotein), in addition to reduced high-density lipoprotein levels (Shattat, 2012). An increase in total cholesterol levels can lead to the risk of atherosclerosis (Owne *et al.*, 2015). The prevalence of dyslipidemia in adults aged ≥25 years in Indonesia was about 36% (33.1% for men and 38.2% for women) (Chao-Feng Lin *et al.*, 2018). Hyperlipidemia is one of the leading causes of cardiovascular diseases (Shattat, 2012). Green beans act as antioxidants, antidiabetics, diuretics, and antibacterials (Ratnayani & Puspawati, 2016; Raffaella *et al.*, 2018; Ramadhani *et al.*, 2020). Green beans contain glycoproteins, trypsin inhibitors, hemagglutinin, β-sitosterol, stigmasterol, allantoin, inositol, leukopelargonidin, quercetin, pelargonidin, cyanidin, kaempferol, petunidin, delphinidin, malvidin, and myricetin (Rahmawani, 2012). The chemical content of green bean extract includes alkaloids, carbohydrates, kumara, protein, amino acids, phenols, saponins, steroids, tannins, and terpenoids (Pascal *et al.*, 2017). In addition to their antioxidant properties that act as LDL reducers in the body, phytosterol components in green beans, such as stigmasterol, can reduce cholesterol levels by inhibiting HMG-CoA reductase, which will reduce LDL cholesterol synthesis. They can also increase HDL cholesterol levels. HDL cholesterol will carry excess LDL cholesterol in the bloodstream back to the liver so that HDL cholesterol prevents cholesterol deposition in the bloodstream, protecting blood vessels from the atherosclerosis process. Stigmasterol is the phytosterol in the ethanol extract of green beans as tested and analyzed by GC-MS (Sri Wahyuni & Ni Luk, 2016). *P. Vulgaris* showed to be an effective anti-hypercholesterolemic agent in ethanol extracts, but further research is needed from several extracts. Therefore, the researchers wanted to

explore the activity of *P. Vulgaris* extracts as anti-hypercholesterolemic agents.

Material and methods

Chemical material

The materials included are aqua dest, pulvis gummi arabicum as a suspending agent, n-hexane, ethyl acetate, 96% ethanol, high cholesterol feed (quail egg yolk, goat fat, cooking oil), propylthiouracil, simvastatin as a comparison drug, n-hexane, ethyl acetate, chloroform, hydrochloric acid, concentrated sulfuric acid, Dragendorf reaction, Mayer reagent, Bouchardat, magnesium powder, amyl alcohol, gelatin solution, iron (III) chloride, Liebermann Burchard reagent, sodium hydroxide, 10% vanillin in concentrated sulfuric acid, ether, 2N hydrochloric acid, and easy touch cholesterol test strips.

Plant material

Green beans were obtained from Cikurubuk Markat, Tasikmalaya, West Java. They were determined by the School of Life Sciences and Technology, Bandung Institute of Technology.

Extraction

The Simplicia of crushed green beans (*P. Vulgaris*) was weighed and then macerated in n-hexane, ethyl acetate, and 96% ethanol. In the first maceration, Simplicia was soaked in solvent until immersed for 24 hours and occasionally stirred (every six hours). The residue was separated from the filtrate and replaced with a new solvent. The process was repeated for 3x24 hours. The liquid extract was then collected in a beaker, and evaporation was carried out using a rotary evaporator to evaporate the solvent and obtain a thick, concentrated extract.

Animals experimental

A total of 36 Rattus male were divided into 9 test groups, namely: control group (not treated), negative group (1% PGA suspension), positive group (simvastatin suspension), group I (rats with induction + 150 mg/kg body weight (bw) n-hexane extract), group II (rats with induction + 300 mg/kg bw n-hexane extract), group III (rats with induction + 150 mg/kg bw ethyl acetate extract), group IV (rats with induction + 300 mg/kg bw ethyl acetate extract), group V (rats with induction + 150 mg/kg bw ethanol extract), group VI (rats with induction + 300 mg/kg bw ethanol extract). All groups except the control group were given high cholesterol diet for 30 days to induce hypercholesterolemia (until the total cholesterol levels were higher than 200 mg/dL), followed by testing for ten days. Before testing, the rats were fasted for eight hours and only given a drink. Then, the total cholesterol level was measured using the easy touch GCU and GCHb Test Strip method.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine significant intergroup differences of each parameter. A p-value <0.05 was considered statistically significant.

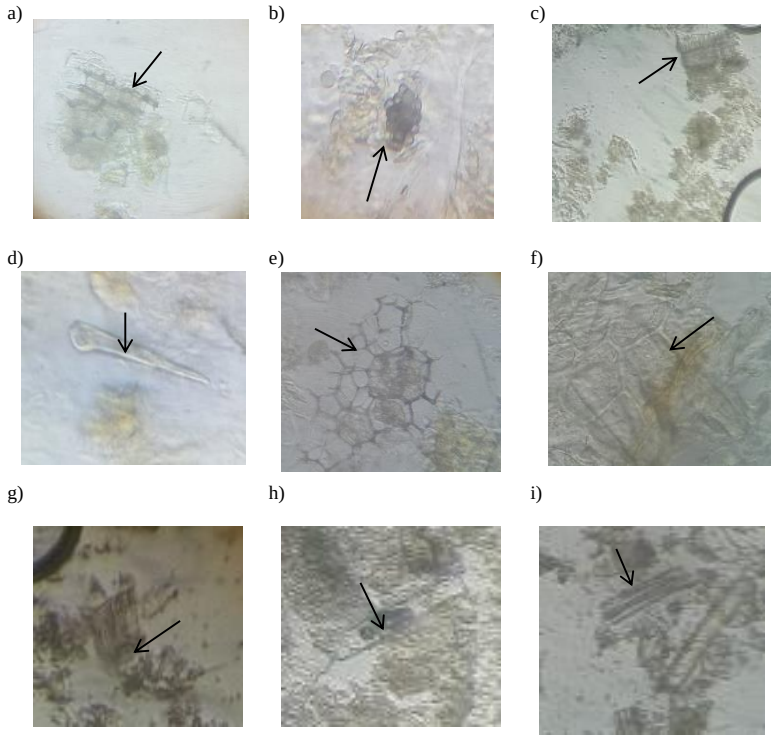
Results

The organoleptic macroscopic observations on the Simplicia of green beans showed a light brown powder with a distinctive odour and no taste. The microscopic observations of green beans Simplicia powder (*Phaseolus vulgaris L*) revealed endocarp, cell fragments containing aleurone grains and oil drops, dotted wooden vessels with ladder thickening, hair covering, endosperm cells, parenchyma cells containing starch grains, sclerenchyma fibres, xylem fibres with calcium oxalate crystal, and xylem fibres (Figure 1).

The results of standardisation of the water content parameters of the green beans Simplicia were carried out using the Azeotropic distillation method (Table I). In general, according to the Indonesian Herbal Pharmacopoeia, the water content of the Simplicia must not exceed 10%. A high water content (more than 10%) can cause the growth of microbes and fungi, thus reducing the quality of the powder and causing changes in enzyme work (Normalisa, 2018). Determination of total ash content is a way to describe the mineral content of Simplicia so that the parameters of the total ash content are related to the purity and contamination of a Simplicia (Tage, 2017). The results of this ash content meet the requirements, which was less than 11%. Determination of drying shrinkage is the percentage limit to the maximum range of compounds lost during the heating process (Tage, 2017). Our results showed an average of 9.33% drying shrinkage.

Table I: Standardisation of Simplicia

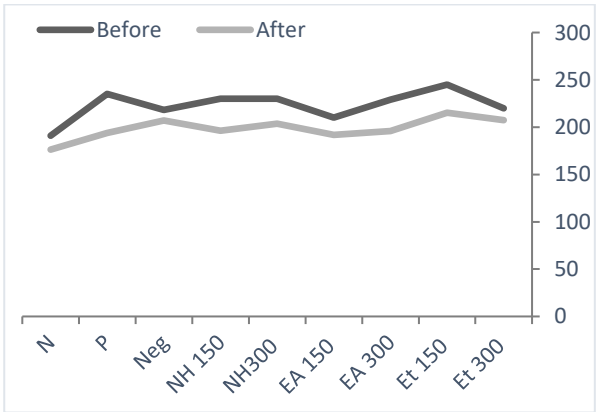
No	Parameter	Average level (%) ± SD
1	Water content	8 ± 0
2	Ash content	9,48 ± 0,112
3	Drying shrinkage	9,33 ± 0,41



a: Endocarp; b: Fragments with cells containing aleurone grains and oil drops; c: Wooden vessels with a thickening of the ladder; d: Conical cover hair; e: Endosperm cells; f: Parenchyma cells containing starch grains; g: Sclerenchyma fibers; h: Xylem fibers with crystalline ca oxalate crystals; i: Xylem fibers.

Figure 1: Microscopic observations of green beans

There were no significant differences in body weight ($p > 0.05$) between groups. However, there were differences in the mean between the groups after induction and after treatment using the test dose (Figure 2).



N: Normal (control); **P**: Positive; **Neg**: Negative group; **NH150**: N-Hexane 150 mg/kg bw; **NH300**: N-Hexane 300 mg/kg bw; **EA 150**: Ethyl acetate 150 mg/kg bw; **EA 300**: Ethyl acetate 300 mg/kg bw; **Et 150**: Ethanol 150 mg/kg bw; **Et 300**: Ethanol 300 mg/kg bw

Figure 2: Average body weight of rats before and after inductions

The 300 mg/kg bw ethyl acetate group rats did not show a significant difference ($p < 0.05$) with the 150 mg/kg bw ethyl acetate group rats, but there were differences in the average total cholesterol levels (Table II).

Tabel II: Effects of green beans on cholesterol levels in rats

Groups	Average of total cholesterol levels (mg/dL)
Normal	125
Positive (2 mg/kg bw simvastatin rats)	176
Negative	238*
150 mg/kg bw n-hexane rats	188.6
300 mg/kg bw n-hexane rats	160
150 mg/kg bw ethyl acetate rats	125.6*†‡
300 mg/kg bw ethyl acetate rats	114.3*†‡
150 mg/kg bw ethanol rats	157
300 mg/kg bw ethanol rats	136*†

* Significant difference with the negative control group ($p < 0.05$)
† significant difference with the positive control group ($p < 0.05$)
‡ Significant difference with the ethanol group 300 mg/kg bw ethanol rat group ($p < 0.05$)

The extract that was the most effective in lowering total cholesterol levels in rats was the 300 mg/kg bw ethyl acetate, showing it had better activity than simvastatin. Indeed, several active compounds of bean extract, such as alkaloids, polyphenols, flavonoids, and steroids, are known to have a cholesterol-lowering activity (Table III).

Tabel III: Phytochemical screening of green beans extract

No	Phytochemical	Reagent	Result			
			Simplicia	n- hexane	Ethyl acetate	Ethanol
1	Alkaloid	Dragendorff	+	+	+	+
		Mayer	+	+	+	+
2	Flavonoids	Mg, HCl, amyl alcohol	+	-	+	+
3	Quinone	NaOH	+	+	+	+
4	Monoterpenoid and sesquiterpenoid	Vanillin 10%	+	+	+	+
5	Steroids	Liebermann	+	+	+	-
	Tritepernoid	burchard	+	-	-	-
6	Saponin		+	-	-	-
7	Polyphenol	$FeCl_3$ 1%	+	-	+	+
8	Tannins	Gelatin 1%	-	-	-	-

Discussion

The active compounds in each solvent were different. The ethyl acetate and ethanol solvent contained flavonoids that were not detected in the n-hexane extract, indicating that the flavonoid components in the green bean extract contain polar flavonoids (Gazali & Nufus, 2019). The ethanol extract did not contain steroid metabolite compounds since steroids are composed of isoprene from long hydrocarbon chains and are non-polar. Ethyl acetate, as a semi-polar solvent, can attract polar and non-polar compounds (Putri *et al.*, 2013). Polyphenol metabolites were not present in the n-hexane extract, indicating that most phenolic compounds were polar compounds that dissolve in polar solvents (Fengel D & Wegener G, 1995). The multilevel maceration method was used for extraction because it could attract compounds based on the polarity of the solvent used. Thus, n-hexane would attract non-polar compounds, ethyl acetate would attract semi-polar compounds, and 96% ethanol would attract polar compounds without any interference of being extracted by other group compounds (Permadi, 2018).

Feeding rats with high-fat foods resulted in weight gain, accompanied by increased serum cholesterol levels (Muzdalifah, 2017). In addition to the high-fat diet that aimed to accelerate the increase in cholesterol levels, propylthiouracil (PTU) was added for the same purpose. The direct effect of PTU-induced hypothyroidism on lipoprotein metabolism is an increase in cholesterol levels, especially LDL-cholesterol caused by the metabolic suppression of LDL receptors, thus increasing LDL levels (Rahayuningsih, 2015). Flavonoid compounds act as hypolipidemic agents and antioxidants; they inhibit oxidative stress and reduce blood cholesterol levels (Wirawan, 2018). The mechanism by which flavonoid compounds can reduce total cholesterol levels and increase the number of LDL receptors in the hepatic cell membrane and extrahepatic tissue is the inhibition of 3-Hydroxy-3-Methyl-Glutaryl-Coenzyme A (HMG-CoA) reductase, which can decrease cholesterol synthesis. They also reduce acyl-CoA cholesterol acyltransferase (ACAT) enzyme activity and cholesterol absorption in the digestive tract (Mutia, 2018). Alkaloid metabolite compounds can reduce cholesterol levels by inhibiting the activity of the pancreatic lipase enzyme, thus increasing fat elimination through faeces. As a result, fat absorption by the liver is inhibited, and fat cannot be converted into cholesterol (Arta *et al.,* 2017).

Other secondary metabolite compounds having a cholesterol-lowering activity are tannins; they inhibit the action of the HMG-CoA reductase enzyme and bind bile acids to the small intestine and phytosterols in steroids by inhibiting the binding of sterol regulatory element-binding protein (SREBP) with sterol regulatory element (SRE), a protein that plays a role in transcription of LDL receptor genes. This inhibition resulted in decreased activity of the enzyme 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase) and decreased chylomicron formation (Naim *et al.*, 2017).

Conclusion

Based on the research results, the activity of 300 mg/kg bw n-hexane extract, 300 mg/kg bw ethyl acetate extract, and 300 mg/kg bw ethanol extract can reduce total cholesterol levels in hypercholesterolemic rats.

The 300 mg/kg bw ethyl acetate was the most effective in lowering cholesterol levels.

References

Agustina KK. (2015).Laboratory animal welfare. *Anim Welf*, 4–11

Artha C, Mustika A & Sulistyawati SW. (2017). Effect of Singawalang Leaf extract effects on LDL levels of hypercholesterolemic male rats. **5**(2), 105–9

Chao-Feng Lin, Ya-Hui Chang, Shih-Chieh Chien, Yueh-Hung Lin & Hung-Yi Yeh. (2018). Epidemiology of dyslipidemia in the Asia Pacific Region. *International Journal of Gerontology*, 12(1), 2-6. https://doi.org/10.1016/j.ijge.2018.02.010

Fengel D & Wegener G. (1995). Kayu: kimia ultrastruktur reaksi-reaksi. Gadjah Mada University Press

Gazali M & Nufus H. (2019). Eksplorasi senyawa bioaktif ekstrak Daun Nipah (Nypa Fruticans Wurmb) asal pesisir Aceh Barat sebagai antioksidan. *Jphpi*, **22**,155–63

Musdalifa N. (2017). Correlation between Body Mass Index and total cholesterol levels in staff and teachers in SMA Negeri 1 Kendari. *e-journal UHO*, **4**(2), 361–7. https://doi.org/10.1109/TPWRD.2018.2825257

Mutia S. (2018). The effect of giving Andong leaf ethanol extract (Cordyline fruticosa (L.) A chev) on total cholesterol and blood triglyceride levels in rats (Rattus norvegicus) hypercholesterolemia. *J Bioleuser*, **2**(2), 29–35

Naim F. (2017). Activity of Dutch Teak Leaf extract on HDL and LDL Cholesterol Levels in Hypercholesterolemic Rats. *Life Sci*, **6**(1), 8

Ahmad, N. (2018). Extraction of natural compounds. Yogyakarta: Deepublish. 39–40

Normalisa. (2018). Effect of ethanol extract of beans (Phaseolus Vulgaris L.) on blood glucose levels and Malondialdehyde levels in diabetic rats. **53**. https://doi.org/10.1017/CBO9781107415324.004

Onwe P, Folawiyo M, Ogah A, Umahi G, Okorocha A & Afoke A. (2015). Hyperlipidemia: etiology and possible control. *IOSR J Dent Med Sci*, **14**(10), 2279–861. https://doi.org/10.9790/0853-1410693100

Pascale R, Bianco G, Cataldi TRI, Kopplin PS, Bosco F & Vignola L. (2018). Mass spectrometry-based phytochemical screening for hypoglycemic activity of Fagioli di Sarconi beans (Phaseolus vulgaris L.). *Food Chem*, **242**, 497–504. https://doi.org/10.1016/j.foodchem.2017.09.091

Permadi A. (2018). Comparison of stratified and unstaged extraction methods of total flavonoids of Ciplukan Herbs by Colorimetry. *Afif Permadi Sutanto Sri Wardatun*, **19**, 7

Putri, W.S, Warditiani, N.K, Larasanty LP. (2013). Phytochemical screening of ethyl acetate extract of mangosteen rind (Garcinia Mangostana L). **5**

Rachmawani NR. (2017). The effect of beans (Phaseolus vulgaris L.) as alternative therapy Of Type 2 Diabetes Mellitus. *Majority*, **6**(1), 71–6

Pascale R., Bianco G., Cataldi, TRI., Kopplin PS., Bosco F., Vignola L., Uhl J., Lucio M. & Milella L.. (2018). Mass spectrometry-based phytochemical screening for hypoglycemic activity of Fagioli di Sarconi beans (Phaseolus vulgaris L.). *Food Chemistry*, **242**, 497-504. https://doi.org/10.1016/j.foodchem.2017.09.091

Ramadhani UP, Chandra B & Rifai H. (2020). Overview of phytochemistry and pharmacology of chickpeas (Phaseolus Vulgaris). *World journal of pharmacy and pharmaceutical sciences*, **9**(9), 442-461

Rahayuningsih Nur & Tita Nofianti. (2015). The Antihyperlipidemia Effect of Strawberry Extract (Fragraria X Ananassa Duchesne). *Jurnal Kesehatan Bakti Tunas Husada*, **13**(1), 213–27

Ratnayani K & Puspawati NR. (2016). Antioxidant and Activity Study Antibacteries on Enzimatic Hydraulic from Protein Kecambah Kacang Komak, Nut Gude And Beans. *Proceeding Semnastect*

Shattat G. F. (2014). A Review Article on Hyperlipidemia: Types, Treatments and New Drug Targets. *Biomed Pharmacol J*, **7**(2)

Sri Wahjuni & Ni Luh Rustini P. (2016). Giving ethanol extract of Beans (Phaseolus Vulgaris L.) to lower total cholesterol, Low Density Lipoprotein (Ldl) And Increase High Density Lipoprotein (Hdl) In Wistar Rats on a High Fat Diet. 103–9

Tage, Novi. (2017). Standardization parameters of fresh plant Simplicia and ethanol extract of beans (Phaseolus vulgaris L.) from Three Different Regions. *Univ Nusant PGRI Kediri*, **1**, 1–7. Available from: http://www.albayan.ae.

Wirawan W. (2018). Est of the effectiveness of Bay Leaf Fraction on total cholesterol levels of hypercholesterolemia-Diabetes male white rats. *J Mandala Pharmacon Indones,* **4**(1), 74–82. https://doi.org/10.35311/jmpi.v4i1.27