

Total Phytosterols Content in Long Beans (*Vigna sinensis*) and Peanut (*Arachis hypogaea*) Seeds

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Abstract

Long beans (*Vigna sinensis* L., Leguminoceae) and peanut (*Arachis hypogaea* L., Leguminoceae) seeds contain phytosterols which are used as an anti-cholesterol empirically. It was suggested that phytosterols can replace cholesterol in the metabolism pathways because of the structure similarity of phytosterols and cholesterol. This study was aimed to determine the total phytosterols content in long beans and peanut seeds.

Total phytosterols content was determined by colorimetric method that measures the green-colored compounds of complex of sterols and Liebermann Buchard reagents. The results showed that total phytosterols content in 100 g of long beans and peanut seeds was 47.62 ± 0.33 and 248.33 ± 1.98 mg, respectively. Total phytosterols content was significantly different ($p = 6.7 \times 10^{-9}$). The difference of total phytosterol content caused the anatomical structure of different plant tissues. Peanut seeds can be used as nutraceutical to meet daily phytosterol needs.

Keywords: Anti-cholesterol, colorimetric, Liebermann Buchard, nutraceutical, daily phytosterol needs.

Introduction

There are more than 40 well-identified and studied phytosterols in plants¹². The phytosterol content increases during the seed germination which is caused by intensive membrane biosynthesis. Phytosterols in meristematic tissue and seeds play a role in cellular proliferation and differentiation⁸. The biological functions of phytosterols in animals are antioxidant capabilities¹³, anticarcinogenic properties²⁰, immune regulation³ and lower blood cholesterol to decrease the risk of heart disease²¹. Phytosterols are the powerful nutraceuticals in lowering cardiovascular disease risk by decreasing the incorporation of cholesterol into micelles¹⁰, thereby decreasing absorption and increasing excretion of cholesterol¹⁴.

Long beans (*Vigna sinensis* L.) and peanuts (*Arachis hypogaea* L.) are Leguminoceae which contain phytosterols. Long beans production was 605.5 tons in 2015⁴ while peanut seeds production was 450.7 tons in 2014⁷. The high production showed the high consumption

of Indonesian society. The aim of this study was to determine total phytosterol content in long beans and peanut seeds and to determine whether the two plants can be used as nutraceutical.

Material and Methods

Materials: Long beans and peanut seeds, aged 1-3 months, were collected from Experimental Farm of Manoko Medicinal Plant, District of West Bandung, West Java, Indonesia, in July 2016. They were identified by School of Biological Sciences and Technology, Bandung Institute of Technology, Indonesia with No. 1125/II.CO2.2/PL/2016. All chemical reagents are of analytical grade (Merck), including the 95% phytosterols standard (Jiatian Biotechnology, Xi'an, China).

Moisture content determination: Each of 5 g of long beans and peanut was dried on 105°C for 5 h at atmospheric pressure, then weighed. Drying and weighing were continued every 1 h until a constant weight¹⁸.

Steroid qualification test: Each of 5 g of long beans and peanut was grinded with 10 mL of chloroform, then add 3 drops of concentrated sulfuric acid followed by 3 drops of acetic anhydride. Positive steroid was shown by alteration color to violet blue and finally green⁹.

Phytosterols Extraction: Each of 100 g of long beans and peanut seeds was extracted with 200 mL of *n*-hexane:ethanol (82:18) for 24 hours at 25 °C. The extract was filtered, then the residue was re-extracted twice with 200 mL of fresh same solvent for 24 hours. All extracts were concentrated by rotary vaporator at 40 °C, then saponified until pH 10.0 with 26.73 M KOH solution. The unsaponified phases were separated with *n*-hexane, then the crude sterols extracts were concentrated¹⁶.

Quantification of total phytosterol content: Liebermann-Buchard (LB) reagent was consisted of the acetic anhydride which was cooled for 30 min, then add concentrated sulfuric acid in the ratio 10:1. Each of 50 mg of crude extract was dissolved in 25 mL of chloroform. The solution (1 mL) was added with 2 mL of LB reagent and chloroform in 5 mL volumetric flask. The mixture was incubated for 5 min, then measure the absorbance. Total phytosterols content was calculated from the linear regression in the calibration curve¹⁶.

Statistical analysis: The results were presented as the mean \pm standard deviation (SD). Statistical analysis was

conducted by one way ANOVA followed by t-test with statistically significant at $p < 0.05$.

Results and Discussion

The moisture content showed the water and volatile compound inside the dried plant material⁵. The moisture content of long beans ($4.1 \pm 0.02\%$) was higher than peanut seeds ($2.3 \pm 0.03\%$). These values met the criteria i.e. 10% ⁵. It was due to differences of used plant parts. The part of long beans was whole pods while peanuts were seeds. Seeds have lower moisture content than pods because of more dried nature⁵. Qualitative test showed that long beans and peanut seeds contain steroids. It was observed from color alteration from yellowish to green solution of complex of phytosterols and LB reagent⁹. These results were in accordance with the literature¹⁹.

Maceration method was chosen to phytosterols extraction due to thermolabile phytosterols. The solvent was a mixture of *n*-hexane and 70% ethanol (82:18), which is non polar to maximize the phytosterols extraction. The pH extract of long beans ($\text{pH } 4.1 \pm 0.2$) was more acidic than peanut seeds ($\text{pH } 5.2 \pm 0.1$). The acid extracts suggested that extracted phytosterols from long beans and peanut seed were bounded form of phytosterols of fatty acid or cinnamic acid because non polar solvent can not dissolve free form¹. Acid extract needed more kalium hydroxide solution to saponification. The aim of saponification was to separate the unsaponified and saponified phase⁹. The unsaponified phase was evaporated to obtain concentrated extract of long beans and peanut seeds i.e. $4.617 \pm 0.231\%$ and $27.765 \pm 0.271\%$ respectively. The results showed that the extracted compounds in peanut seeds was higher than long beans.

The optimal time for green color formation of the sterols and LB reagents was 5 min with maximum absorbance at 626.7 nm ¹⁷. The result of validation method (table 1) met the criteria. It means the instrument response was comparable to the analyte concentrations with good accuracy and precision⁶.

Table 1
Validation Result of Quantitative Analysis

Parameter	Results ¹⁷	Reference ⁶
Linearity (correlation coefficient)	0.9992	0.990
Accuracy (% recovery)	96.7-106.9%	80-110%
Precision (coefficient of variance)	1.553%	less than 2%
Limits of detection (LOD)	0.915 $\mu\text{g/mL}$	-
Limits of quantitation (LOQ)	3.51 $\mu\text{g/mL}$	-

Total phytosterols content in 100 g of long beans and peanut seeds was $47.62 \pm 0.33 \text{ mg}$ and $248.33 \pm 1.98 \text{ mg}$ respectively. The total phytosterols content of peanut seeds was higher than long beans. It was in accordance with the literature that peanut seeds are rich in phytosterols compared to vegetables such as long bean¹⁹. There was statistically significance on total phytosterols content ($P = 6.7 \times 10^{-9}$). The difference of phytosterol amount caused the anatomical structure of different plant tissues¹⁵. Peanut seeds are germinated part of the plant. If the total phytosterol content is higher than the long bean pods which consist of seeds and flesh, so at the same weight it has different seed weight.

Phytosterol synthesis appears during the seed formation and germination which provide supply for the growth of new cells and young shoots⁸. Eating habits affect the phytosterols intake, generally ranging from 200 to 300 mg/day ². Eating 100 g of peanut seeds was sufficient for daily phytosterol needs, compared to have eating 500 g of long beans. Peanut seeds can be used as nutraceutical to meet daily phytosterol needs.

Conclusion

Peanut seeds can be used as nutraceutical to meet daily phytosterol needs.

References

- Akhisa T. and Kokke W., Naturally occurring sterols and related compounds from plants, In Patterson G.W. and Nes W.D., eds., *Physiology and Biochemistry of Sterols*, American Oil Chemists' Society, Champaign, IL, 172-228 (1991)
- Andersson S.W., Skinner J., Ellegård L., Welch A.A., Bingham S., Mulligan A., Andersson H. and Khaw K.T., Intake of dietary plant sterols is inversely related to serum cholesterol concentration in men and women in the EPIC Norfolk population: A cross-sectional study, *Eur J Clin Nutrition*, **58(10)**, 1378-85 (2004)
- Brüll F., Mensink R.P., van den Hurk K., Duijvestijn A. and Plat J., TLR2 activation is essential to induce a Th1 shift in human peripheral blood mononuclear cells by plant stanols and plant sterols, *J Biol Chem*, **285**, 2951-58 (2010)
- Central Bureau of Statistics, Produksi Kacang Tanah Menurut Provinsi (ton), 1993-2015, available at <https://www.bps.go.id/linkTableDinamis/view/id/874>, cited on August 1st 2017 (2017)
- General Directorate of Food and Drug Control, *Materia Medika Indonesia*, volume 5, Department of Health of Republic Indonesia, Jakarta (1989)
- EMA, ICH Topic Q 2 (R1), Validation of Analytical Procedures: Text and Methodology, in International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, European Medicines Agency, London (2008)

7. General Directorate of Horticulture, Ministry of Agriculture, Statistik produksi hortikultura tahun 2014, available at <http://hortikultura.pertanian.go.id/wp-content/uploads/2016/02/Statistik-Produksi-2014.pdf>, cited on August 1st 2017 (2017)
8. Grunwald C., Steroids, In Encyclopedia of Plant Physiology, Pirson A. and Zimmermann M.H., eds., **8**, 221-39 (1980)
9. Harborne J.B., Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd ed., Chapman and Hall, London, 302 (1998)
10. Jesch E.D. and Carr T.P., Sitosterol reduces micellar cholesterol solubility in model bile, *Nutr Res*, **26**, 579-84 (2006)
11. Jesch E.D. and Carr T.P., Food Ingredients That Inhibit Cholesterol Absorption, *Prev Nutr Food Sci*, **22(2)**, 67-80 (2017)
12. Law M., Plant sterol and stanol margarines and health, *British Med J*, **320**, 861-64 (2000)
13. Loizou S., Lekakis I., Chrousos G.P. and Moutsatsou P., Beta-Sitosterol exhibits antiinflammatory activity in human aortic endothelial cells, *Mol Nutr Food Res*, **54**, 551-58 (2010)
14. Miettinen T.A., Vuoristo M., Nissinen M., Järvinen H.J. and Gylling H., Serum, biliary and fecal cholesterol and plant sterols in colectomized patients before and during consumption of stanol ester margarine, *Am J Clin Nutr*, **71**, 1095-102 (2000)
15. Piironen V., Toivo J., Puupponen-Pimi R. and Lampi A.M., Plant sterols in vegetables, fruits and berries, *J Sci of Food and Agricult*, **83**, 330-337 (2003)
16. Saptarini N.M., Indriyati W. and Shalihah A., Colorimetric Method for Total Phytosterols Content Analysis in Soybean (*Glycine max*), Soymilk and Soy Yoghurt, *J Chem and Pharm Res*, **8(4)**, 1458-64 (2016)
17. Saptarini N.M., Herawati I.E. and Hernawati N.F., Total Phytosterols Content in Roselle (*Hibiscus sabdariffa*) Calyx from Subang and Bandung, *Res J Pharm, Biol and Chem Sci*, **8(1S)**, 174-78 (2017)
18. The United States Pharmacopeial Convention, US Pharmacopeia, 32th ed., United States, Rockville, MD (2008)
19. Valsta L.M., Lemström A., Ovaskainen M.L., Lampi A.M., Toivo J., Korhonen T. and Piironen V., Estimation of plant sterol and cholesterol intake in Finland: Quality of new values and their effect on intake, *British J Nutr*, **92(4)**, 671-8 (2007)
20. Woyengo T.A., Ramprasath V.R. and Jones P.J.H., Anticancer effects of phytosterols, *Eur J Clin Nutr*, **63**, 813-820 (2009)
21. Xu J., Kochanek K., Murphy S. and Tejada-Vera B., Deaths: final data for 2007, *Natl Vital Stat Rep*, **58**, 1-136 (2010).