INTRODUCTION

Peas (Pisum sativum L.) and red beans (Phaseolus vulgaris L.) are member of Leguminosae or Fabaceae family. Peas contain folic acid, carbohydrates, protein, Vitamin K, and phytosterols, especially β-sitosterol,[1] while red beans contain protein, fat, carbohydrate, fiber, and other compounds,[2] such as β-sitosterol, i.e., 86.5 mg/100 g.[3] The most abundant phytosterols in nature are β-sitosterol, campesterol, stigmasterol, and cycloartenol.[4] Although phytosterol has a similar structure to cholesterol, it has the opposite function and used as an anticholesterol. Phytosterols act to reduce cholesterol absorption in the intestine lumen by increasing fecal cholesterol excretion.[5] These phytosterols are generally recognized as safe, and the dietary intake to achieve significant cholesterol plasma reductions (5–15%) is 2 g per day.[6] Quantitative analysis of phytosterol can be done by colorimetric method between phytosterol and Liebermann–Burchard reagent.[7] Validation of colorimetry methods on the determination of total phytosterol content met the International Conference on Harmonization (ICH) criteria.[8] The aim of this study is to determine total phytosterol content in red beans and peas, which are planted in Bandung, and then determined the possibility as a nutraceutical source.

MATERIALS AND METHODS

Materials

Red beans and peas, aged 1–3 months, were collected from Experimental Farm of Manoko Medicinal Plant, District of West Bandung, West Java, Indonesia, in July 2016. Plant identification was conducted by Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics and Science, Universitas Padjadjaran, Indonesia with No. 012/HB/04/2016. All chemical reagents were analytical grade and purchased from Merck (Germany), except 95% phytosterol standard (Jiatian Biotechnology, Xi’an, China).

Determination of Water Content

Each of red beans and peas was dried on 105°C at atmospheric pressure for 5 h and then weighed.
Drying and weighing were continued, every 1 h, until a constant weight.\(^9\)

**Steroid Qualification Test**

Each of red beans and peas was ground with chloroform, and then, concentrated sulfuric acid and acetic anhydride were added sequentially. Positive steroid was showed by alteration color to violet blue and finally green.\(^7\)

**Extraction of Phytosterols**

Each of red beans and peas was extracted with \(n\)-hexane: ethanol (82:18) at 25°C for 24 h. The residues were reextracted with the same fresh solvent for 24 h. All extracts were concentrated by rotary evaporator and then saponified until pH 10.0 with 26.73 M KOH solution. The unsaponifed phases were extracted with \(n\)-hexane, and then, the crude sterols extracts were concentrated.\(^10\)

**Analysis of Total Phytosterol Content**

Liebermann–Burchard (LB) reagent was consisted of the acetic anhydride which cooled for 30 min and then added concentrated sulfuric acid in the ratio 10:1. Each of 50 mg of crude sterol extract was dissolved in 25 mL of chloroform. Each of 1 mL of solution was added with 2 mL of LB reagent and fulfilled with chloroform in 5 mL volumetric flask. The mixture was incubated for 5 min, and then, the absorbance was measured. The linear regression of the calibration curve was used to calculate the total phytosterol content.\(^10\)

**Statistical Analysis**

Data were presented as the mean ± standard deviation. The statistically significant difference was expressed if \(P < 0.05\) was conducted by one-way ANOVA followed by \(t\)-test.

**RESULTS AND DISCUSSION**

The Leguminosae is being found everywhere, except Antarctica and the high Arctic. The herbaceous plants and shrubs are predominant outside the tropics.\(^11\) Red beans and peas are herbaceous plants, so Lembang is good place for this legume plantation. It is located between 1.31 and 2.08 m above sea level. The average temperature is 25.2°C and average rainfall is 2137 mm annually.\(^12\) The water content of red beans and peas was 2.0% and 2.6%, respectively. These results met the criteria, i.e. 10%.\(^13\)

The qualitative test of steroids showed that red beans and peas containing steroids, which observed through color alteration to green solution due to the reaction between steroids and LB reagent. These results are in accordance with the literature.\(^1,3\)

Figure 1: Calibration curve of phytosterols

Maceration is chosen because of the high yield and preserves the secondary metabolites. The principle of maceration is the slow transfer of solutes to the solvent until equilibrium.\(^14\) Mixed solvent (\(n\)-hexane: ethanol, 82:18) was chosen to phytosterols extraction because of highest yield compare to other mixed solvents.\(^15\) Saponification was aided by heating to hydrolyze the ester bonds between fatty acids and sterols to form two miscible phases.\(^16\) Crude sterol extract of red beans and peas was 3.33% and 4.07%, respectively.

Validation method which was done in the previous study was met the ICH criteria.\(^17\) Correlation coefficient was 0.9992 [Figure 1] which was met with the ICH criteria.\(^17\) Total phytosterol content in 100 g of red beans and peas was 112.94 ± 0.68 and 82.88 ± 0.42 mg, respectively. The ratio of total phytosterol content to crude sterol extract was 3.38% for red beans and 2.03% for peas. Total phytosterol content of red beans was higher than peas, which was significantly different (\(P = 3.34 \times 10^{-7}\)), because of different species.

These results were lower than literature, i.e., 135 mg/100 g peas and 127 mg/100 g red beans.\(^18\) This is due to differences in the growth climate of the samples. Climatic factors do affect the distribution between sterols and stanols. This is indicated from the lowest phytosterol content which was found in the year with lowest average temperature and highest rainfall.\(^16\) Red beans and peas in this study were collected on July 2016, with lowest rainfall was 195 mm in June and highest rainfall was 260 mm in May. Lembang has significant rainfall in most of the month, with short dry seasons. This condition was classified as Am according to Köppen and Geiger classification.\(^12\) High rainfall causes low total phytosterol content in this legume.

Low doses (60 mg/day) of phytosterols decrease the symptoms of benign prostatic hyperplasia in clinical trials\(^19\) and have a positive impact on the human immune system.\(^20\) Large amount of vegetable consumption plays an important role in increasing the
people’s phytosterols intake. The daily phytosterol intake from natural sources normally is between 200 and 300 mg/day. Eating 200 g of red beans, which is equal to 225 mg of total phytosterols content, was sufficient for daily phytosterol needs, so red beans can be used as a nutraceutical source.

CONCLUSION

Red beans are potentially used as nutraceutical to meet daily phytosterol needs.

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