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Short Communication: Optimization of extraction of sulfhydryl compounds from several legumes seeds in Indonesia with various ethanol concentrations

SRI WARDATUN[1,♥], YAHDIANA HARAHAP[1,♥♥], ABDUL MUN'IM[2,♥♥♥], NOORWATI SUTANDYO[3,♥♥♥♥]

[1]Laboratory of Bioavailability and Bioequivalence, Faculty of Pharmacy, Universitas Indonesia. Kampus UI, Depok 16242, West Java, Indonesia. Tel.: +62-21-7270031, Fax.: +62-21-7863433, ♥email: sri.wardatun07@gmail.com, ♥♥email: yahdiana03@yahoo.com

[2]Laboratory of Phytochemistry, Faculty of Pharmacy, Universitas Indonesia. Kampus UI, Depok, 16424, West Java, Indonesia. Tel. +62-21-7270031, Fax.: +62-21-7863433, ♥♥♥email: abdul.munim61@ui.ac.id

[3]Dharmais Cancer Hospital. Jl. Letjen S. Parman No. 84-86, Palmerah, Jakarta Pusat 11420, Jakarta, Indonesia. Tel./fax.: +62-21-5681570, ♥♥♥♥email: noorwatis3@yahoo.com

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Abstract. *Wardatun S, Harahap Y, Mun'im A. 2020. Short Communication: Optimization of extraction of sulfhydryl compounds from several legumes seeds in Indonesia with various ethanol concentrations. Biodiversitas 21: 1060-1064. Leucaena leucocephala* (Lam.) de Wit (*petai Cina*), *Parkia speciosa* Hassk. (*petai*), and *Archidendron jiringa* (Jack) I.C. Nielsen (*jengkol*) seeds extracts contain sulfhydryl compounds and have various therapeutic properties. The main objective of this research was to compare the effect of various ethanol concentration on the yield of extract and levels of sulfhydryl compounds from leguminous seeds. Dried seeds were macerated at room temperature (25°C) with a ratio of solids and solvents was 1:10. The solvent concentrations used were 30%, 50%, 70% and 96% ethanol. The yield of extract was expressed as the ratio of the weight of extract to the weight of dried seeds, the level of sulfhydryl compound was expressed as reduced L-glutathione (GSH) equivalent. The level of sulfhydryl compounds was determined by Ellman reagent and further analyzed using a spectrophotometer at 411 nm wavelength. Stink bean produced the highest yield of extract, while the highest level of sulfhydryl compounds was obtained from *petai cina* seed extract. The concentration of ethanol gave a significant difference to the yield of extract and the level of sulfhydryl compounds. The results showed that ethanol concentration affected the efficiency of the extraction of total sulfhydryl compounds and the yield of the extract on leguminous seeds.

Keywords: Leguminous seed, solvent, sulfhydryl compound, yield of extract

INTRODUCTION

Leucaena leucocephala (Lam.) de Wit is a tropical leguminous tree in the family of Leguminosae found in Indonesia and the other tropical areas (Syamsudin et al. 2010; Verma 2016). The common name of *L. leucocephala* is *petai cina* or *lamtoro* (Indonesia) and white lead tree (English) (Soetjipto et al. 2019). *Parkia speciosa* Hassk. or stink bean in Indonesia and Malaysia is known as *petai* (Tocmo et al. 2016; Chhikara et al. 2018). *Archidendron jiringa* (Jack) I.C. Nielsen is locally as known as *jering*" as well as *jengkol* in Indonesia. *P. speciosa* and *A. jiringa* were the other tropical leguminous tree in the family of *Leguminosae*. Seeds from these legumes often used as food sources and have several health benefits. *L. leucocephala* used as anthelmintic, antidiabetic (Abdelhady and Abdallah 2016; She et al. 2017), anticancer and anti-metastasis (She et al. 2017), antioxidant and tyrosinase inhibitor (Li 2012). *P. speciosa* seeds have antioxidant, antiproliferative and hypoglycemic activity (Chhikara et al. 2018). *A. jiringa* seeds used as antiulcerogenic and have pancreatic lipase inhibitory activity (Abdel et al. 2012; Seyedan et al. 2015). Seeds of these plants have distinctive aroma suggesting the presence of sulfur/thiol compounds (Suvachittanont et al. 1996). The sulfur compound in seed could be cysteine, and their derivatives such as glutathione, djenkolic acid and thiazolidine-4-carboxylic acid (TCA) which is often called thioproline (Suvachittanont et al. 1996). Thiol compound has functional groups of sulfur and hydrogen atoms (-SH) often called sulfhydryl compounds (Baron and Sochor 2013). Sulfhydryl compounds in the body play the role of glutathione (ϒ-glutamyl-cysteinyl-glycine; GSH) and have the function of antioxidants to scavenge reactive oxygen and nitrogen as well as a cofactor in several types of enzymes (Gaucher et al. 2018; Lushchak 2012).

Extraction is the separation of the soluble plant metabolites leaving insoluble residues (Azwanida 2015). The extraction process is an important part of taking active compounds (Gupta et al. 2012; Azmir et al. 2013). The extraction of sulfhydryl compounds from leguminous seeds can be done optimally if the solvents used are suitable. The choice of solvent will determine the type of compound being extracted from the sample (Azwanida 2015). A number of leguminous seeds contain water-soluble sulfhydryl compounds. The extraction of sulfhydryl compounds from Leguminous seeds has been done by using water as a solvent (Suvachittanont et al. 1996). The use of water as a solvent has no impact on the environment but it dissolves undesired protein and polysaccharides (Shi et al. 2003; Plaza and Turner 2015). The presence of

protein and polysaccharides results in concentration polarization and reduced filterability when the filter membrane used for purification (Shi et al. 2003). Using water as a solvent in extraction might be energy demanding in cases where water needs to be removed by evaporation (Plaza and Turner 2015). Ethanol is an organic and non-toxic solvent, and most widely used for extraction (Alam and Bristi 2013). High concentrations of ethanol increase the cost of extraction (Shi et al. 2003). Solvent concentration plays an important role in the efficiency and affects the quantity and secondary metabolites composition (Pandey and Tripathi 2014; Sun et al. 2015). The objective of this study was to optimize the extraction conditions by varying ethanol concentration to maximize the yield of extract and levels of sulfhydryl containing-compounds from leguminous seeds.

MATERIALS AND METHODS

Chemicals and samples

Reduced L-Glutathione as standard and 5-5 dithiobis-2-nitrobenzoic acid (DTNB; Ellman's reagent) from Sigma Aldrich, ethanol from Merck, potassium phosphate monobasic, sodium hydroxide (Merck). All chemicals used were analytical grade purchased from Merck.

Fresh seeds of *P. speciosa* and *A. jiringa* were purchased from the local market in Bogor, Indonesia**.** The seeds were sliced (3-4 mm) and dried in an oven at 40^0C for 86 hours. *L. leucocephala* seeds were collected from Ciomas, Bogor-Indonesia in December 2018 then dried in an oven at 40°C for 86 hours. The samples were characterized at The Center for Plant Conservation, Botanic Gardens, Indonesia. All samples were mature seeds. The weight of dried seeds was determined after air drying and weighed until a constant weight was obtained. Dried weights of these seeds were found to be 38.56; 27.58 and 29.71% of fresh weight for *L. leucocephala, P. speciosa* and *A. jiringa*, respectively

Preparation of extract

Dried seeds were crushed to powders. The powder of seeds (50 grams) was macerated with 250 mL different concentrations of ethanol (30%, 50%, 70%, and 96%) for 24 hours, respectively. Remaceration was done in 2x24 hours with 150 mL ethanol and 100 mL ethanol (Azwanida 2015). The ethanol extract was separated from the residual seed powder. The filtrate was evaporated with a rotary evaporator. Water content in the crude extract was determined gravimetrically. Extraction was done in triplicates.

The yield of extract

The dried extract was weighed to obtain the yield of extract from the following equation:

The yield of extract= $W_{extract}/W_{dried\ seeds.\ We\ extract}$ is the weight of the extract. $W_{dried\ seeds}$ are the weight of dried seed (Xu et al. 2017).

Analysis of total sulfhydryl compound in extract

Analysis of total sulfhydryl compound was carried out according to Ellman 1959; Haque et al. 2003; Khan et al. 2012. The extract was weighed and dissolved with distilled water in a conical flask then filtered with a syringe filter. In brief, 2300 µL of phosphate buffer (K_2HPO_4/NaOH, 200 mM, pH 7.6) were mixed with 200 µL sample. Then, 500 µL of DTNB (5,5'-dithio-bis (2-nitrobenzoic acid) solution (1 mM in the same phosphate buffer) were added and the mixture was shaken using vortex in 10 seconds and kept at room temperature (20°C) for 2 minutes. The absorbance of the filtrate of the sample was measured at λ_{max} 411 nm against a blank containing buffer instead of the DTNB solution using Jasco V-730 UV/VIS double beam spectrophotometer. Total sulfhydryl compound was determined using the standard curve of reduced L-glutathione (GSH) obtained from different concentrations of GSH (50, 100, 150, 200, 250, and 300 µg/mL). The levels of total sulfhydryl compound expressed as GSH equivalent (µmol/100 g dry seeds).

Standard curve of reduced glutathione

Phosphate buffer solution (2300 µL) of pH 7.6 was added to 200 µL of 50, 100, 150, 200, 250, and 300 µg/mL solutions of GSH, followed by the addition of 500 µL of 1 mM DTNB stock solution. The mixture was shaken using vortex in 10 seconds and kept at room temperature (20^0C) for 2 minutes. The absorbance was measured at λ_{max} 411 nm. The standard curve was constructed between concentration with absorbance. Straight-line was drawn. The Coefficient regression was 0.9991.

Statistical analysis

All experiments were carried out in triplicates. Results were presented as average±SD. The significant difference between treatment was analyzed using the ANOVA. $P<0.05$ was considered as the level of significance.

RESULTS AND DISCUSSION

The yield of extract

The yield of extract in different concentrations of the solvent had been varied depending on the concentration of ethanol (Figure 1). The yield of *P. speciosa* extract derived from 30% ethanol and 96% ethanol did not show a significant difference, and the yield of extract from 50% and 70% ethanol showed no significant difference. The yield of *P. speciosa* seed extract from 30% ethanol and 96% ethanol, when compared to the yield of *P. speciosa*, extract from 50% ethanol and 70% ethanol showed significant differences.

A solvent of 96% ethanol results in the lowest yield of extract, while 30% ethanol results in the highest yield of extract at *A. jiringa*. Ethanol concentrations of 50% and 70% did not show a significant yield of *A. jiringa* extract. The concentration of 30% ethanol and 96% ethanol showed a significant effect on the yield of extract. The ethanol concentration of 96% results in the lowest yield of extract, while 30% ethanol results in the highest yield of *L.*

leucocephala extract, while ethanol concentrations of 30% and 50% did not show a significant yield of *L. leucocephala* extract. Statistical analysis showed that ethanol concentrations of 96%, 70%, and 30% ethanol showed a significant yield of leguminous seeds extract.

Total sulfhydryl compound

Figure 2 showed the total sulfhydryl compound of leguminous seeds expressed as GSH equivalent. The results showed that the total sulfhydryl compound extracted from different ethanol concentrations varied. The ethanol concentration of 30% produced the highest level of total sulfhydryl compound in *P. speciosa* and *A. jiringa* seeds, and 50% ethanol produced the highest total sulfhydryl compound in *L. leucocephala* seeds. The ethanol concentration of 96% ethanol produced the lowest level of total sulfhydryl compound in these leguminous seeds. *L. leucocephala* seeds produced the highest total sulfhydryl compound compared to other Leguminous seeds. Statistical analysis showed that different ethanol concentrations significantly affected the level of total sulfhydryl compounds $P<0.05$).

Discussion

Sulfhydryl compounds can act in the body as glutathione (ϒ-glutamyl-cysteinyl-glycine; GSH). GSH may play a role during oxidative stress and act as an antioxidant (Lushchak 2012). The presence of sulfhydryl compounds can be smelled from its distinctive aroma (Suvachittanont et al. 1996). Solvent has a critical role in the amount of extracted compounds (Pandey and Tripathi 2014). The solvent is mainly selected in terms of availability, cost, inertness, toxicity, environmental fate, solubility, polarity and boiling point. Polarity plays a critical role as the polar solvent is able to extract polar compounds (Maltese et al. 2009). The concentration of solvent could affect viscosity, change the dielectric constant and could produce a difference of polarity solution extract (Shuai and Luterbacher 2016). On the basis of their chemical properties, different compounds are extracted from different polarity solvents (Rauf et al. 2018). Maceration methods are used to avoid the destruction of thermolabile sulfhydryl compounds. The maceration method is the most suitable for extracting the thermolabile compounds (Pandey and Tripathi 2014).

Study on the levels of thiol compound in Leguminous seeds using distilled water as a solvent has been done in Thailand with the following results: *L. leucocephala* (1.5 mmol/100g dried seeds), *P. speciosa* (4.40 mmol/100 g dried seeds), and *A. jiringa* (0.30 mmol/ 100 g dried seeds) (Suvachittanont et al. 1996). Figure 1 showed that different ethanol concentrations produced different amounts of extract. This is in agreement with the result by Shuai and Luterbacher (2016) that varying ethanol concentrations result in the difference in yield of extract (Shuai and Luterbacher 2016). Different ethanol concentrations have different polarities. Water has a higher polarity index than ethanol. Polarity index of water and ethanol was 10.2 and 5.2, respectively (Ramluckan et al. 2014). The addition of ethanol to water caused a decrease in the polarity index of the mixture. The use of mixed solvents allows all compounds to be extracted because of changes in polarity. The use of mixed solvents increases the efficiency of extraction, while the use of a single solvent does not ensure that all of the phytochemical compounds in the plant could be extracted (Rauf et al. 2018). Figure 1 showed that an increase in ethanol concentration causes the yield of the extract to decrease. This can be caused that the compounds in the extract have high polarity, so it was difficult to dissolve in low polarity solvents. *P. speciosa* produced the highest yield of extract in 96% ethanol concentration. This can be caused that *P. speciosa* contain chemical compounds that are more soluble in 96% ethanol (Chhikara et al. 2018).

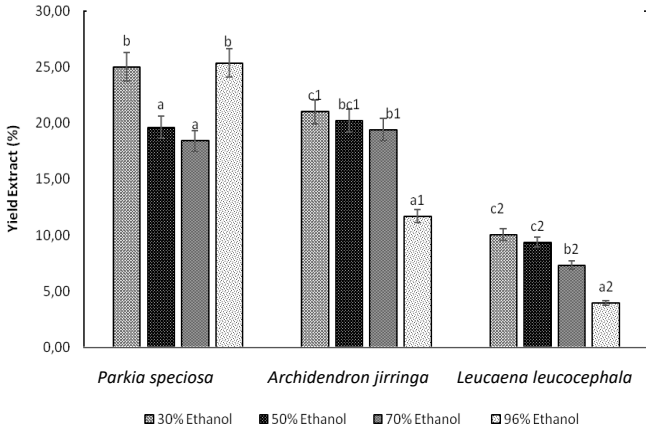

Figure 1. Effect of solvent concentration to yield of leguminous seed extracts

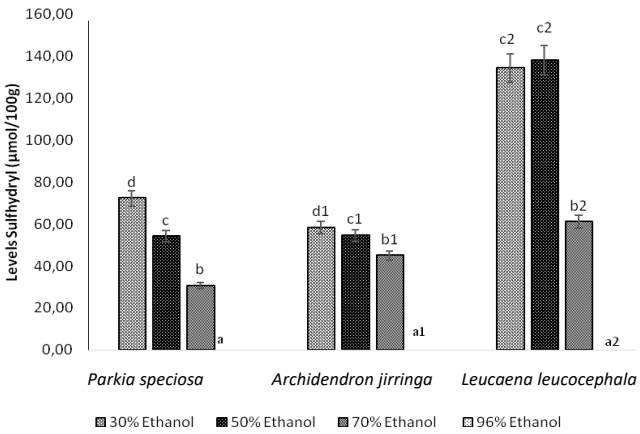

Figure 2. The effect of solvent concentrations on sulfhydryl levels of leguminous seeds

Sulfhydryl compounds are water-soluble and oil-soluble (de Vallle et al. 2008). Water-soluble sulfhydryl compounds could be extracted with ethanol because ethanol was a polar solvent (Shuai and Luterbacher 2016). Differences in ethanol concentration cause differences in the amount of extracted sulfhydryl. Lower ethanol concentrations produced higher levels of extracted sulfhydryl in all leguminous seeds. The concentration of 30% ethanol gave the highest levels of sulfhydryl compounds in all leguminous seeds. The polarity index of 30% ethanol was higher than other ethanol concentrations. Sulfhydryl compounds were polar compounds that were more soluble in 30% ethanol. The 96% ethanol concentration unable to extract sulfhydryl compound hence sulfhydryl compound in 96% ethanol was not detected or below the detection limit. Water-soluble sulfhydryl compounds contain allyl sulfur with carboxylic groups (Tapiero et al. 2004). The ethanol concentration of 96% has a low polarity index, so the solvent was unable to extract sulfhydryl compounds. *P. speciosa* contains cyclic and non-cyclic organosulfides which comprise 36% of the total volatiles. Volatile organosulfides are more soluble in n-hexane than ethanol (Tocmo et al. 2016). The results of this study showed that non-volatile organosulfides are more soluble in low concentrations of ethanol. The content of sulfhydryl compounds of *L. leucocephala* was higher than *P. speciosa* and *A. jiringa*. Sulfhydryl compounds in seeds could be cysteine and their derivates such as glutathione, djenkolic acid, and thiazolidine-4-carboxylic acid. These compounds are soluble in polar solvents (Suvachittanont et al. 1996).

The level of sulfhydryl compounds in Leguminous seeds in Indonesia differs from the levels of sulfhydryl compound in Leguminous seeds from Thailand (Suvachittanont et al. 1996). The plant growth and various cultivation affect on chemical composition (Martins et al. 2016). The use of ethanol for solvents and the fresh or dried samples in this research can affect to levels of sulfhydryl compounds obtained. The drying process may affect the content of available sulfhydryl compounds (Suvachittanont et al. 1996). The evaporation process of the extract using a rotary evaporator at 40^0C may also affect the total sulfhydryl content. The level of total sulfhydryl compounds in the seeds of *L. leucocephala* was the highest compared to other Leguminous seeds. However, this finding differed from the result of Suvachittanont et al. (1996), they used water distilled for extraction and this research used ethanol for extracted. The type solvent and solvent concentration affect the quality, quantity, and composition of secondary metabolite extracted (Pandey and Tripathi 2014). The statistical analysis showed that ethanol concentrations affect the yield of extract and the efficiency of extraction of total sulfhydryl-containing compounds in Leguminous seeds. The best ethanol concentration for extracting sulfhydryl compounds in legumes seeds was 30% ethanol solvent.

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