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FULL PAPER

Extraction of saponins, total soluble solids and antioxidant activity from *Polyscias fruticosa* roots

Chuyen, H.V.

Faculty of Chemical and Food Technology, HCMC University of Technology and Education, Ho Chi Minh City, Vietnam

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Abstract

Polyscias fruticosa is a tropical plant that has been used as medicine and food in Asian countries for centuries. The roots of *Polyscias fruticosa* contain a significant amount of beneficial bioactive compounds including saponins, polyphenols and flavonoids. Although a few reports on the extraction of bioactive from *Polyscias fruticosa* roots have been published, the literature related to the effects of extraction parameters on the recovery of saponins, the predominant bioactive group in this medicinal plant is limited. In this study, the effects of ethanol concentration, temperature, extraction time and liquid-solid ratio on the recovery of total soluble solids (TSS), saponins and antioxidant activity (AA) of extracts from *Polyscias fruticosa* roots were investigated. The yield of saponins, TSS and AA were significantly affected by the extraction conditions. The extraction time of 120 mins resulted in the highest yield of TSS while the extraction time of 90 mins led to the highest recovery of saponins and AA. Total saponin recovery increased along with the increase in ethanol concentration and reached a plateau at 40% of ethanol concentration. In addition, the temperature of 50^{o}C and the liquid-solid ratio of 40/1 (mL/g) were found to be the most favourable conditions for the extraction. The results of this study were applicable for the process development on larger scales to produce saponin-rich products from *Polyscias fruticosa* roots.

1. Introduction

Polyscias fruticosa has been popularly grown in Vietnam and some Asian countries for centuries to be used as traditional medicine as well as an ingredient in foods. Recent studies have shown that *Polyscias fruticosa* contains a significant amount of saponin compounds (including some saponins similar to those found in Korean ginseng (*Panax ginseng*) and some other phytochemicals such as phenolic acids and flavonoids. Those compounds have been reported to have many beneficial bioactivities such as antioxidant, anti-inflammatory activity, and the ability to support diabetes treatment as well as the treatment of some types of cancer (Bernard *et al.*, 1998; Vo *et al.*, 1998). Beneficial bioactive compounds in *Polyscias fruticosa* leaves such as oleanane-type triterpenoid saponins have also recently been found (Luyen *et al.,* 2018) and these bioactive substances also exhibited significant biological activity (Do *et al.*, 2020). Therefore, the economic value of *Polyscias fruticosa* has increased significantly and the commercial cultivation of *Polyscias fruticosa* has also been strongly developed throughout Asian countries.

Although there have been a few studies related to the extraction of bioactive ingredients from *Polyscias fruticosa* roots to produce concentrate or powders, these studies mainly used traditional extraction methods at high temperatures (boiling) with water as the solvent to extract the bioactive constituents (Vo, 1992; Dang, 2016). The disadvantage of the traditional extraction method is that the extraction efficiency is low and the high decomposition of bioactive substances. Recently, Nguyen *et al.* (2020) published the results of a study using ethanol solutions for the extraction of polyphenols and flavonoids from *Polyscias fruticosa* roots. The report showed that the extraction using 90% ethanol as the solvent with the solvent-solid ratio of 1:20 g/mL for 3 h at 30°C resulted in the highest extraction yield of polyphenols, flavonoids and DPPH and ABTS antioxidant capacity. Most of the previous studies have not deeply investigated the influence of technological factors such as solvent type, solvent ratio or extraction temperature and different extraction methods on the extraction efficiency of saponins, the predominant bioactive ingredients, from *Polyscias fruticosa* roots.

*Corresponding author.
Email: *chuyenhv@hcmute.edu.vn*

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Thus, studies on the effect of extraction to determine the appropriate parameters to increase the recovery and reduce the degradation of saponins from *Polyscias fruticosa* roots in order to obtain products with high biological activity are necessary.

In this study, the effects of temperature, extraction time, ethanol concentration and the ratio of solvent to the material on the extraction efficiency of total soluble solid (TSS), saponins and antioxidant activity (AA) of extracts from *Polyscias fruticosa* roots were investigated.

2. Materials and methods

2.1 Materials

Polyscias fruticosa roots (PFR) with the age of 6 years old (the recommended age for harvesting of PFR) (Luyen *et al.*, 2018) were collected from farms in Ho Chi Minh City, Vietnam. The material was then washed thoroughly with clean water, sliced and then freeze-dried to the moisture content of 3.5% and stored at -18^{o}C. The dried material was ground by grinder HR2118 (Philips, Jakarta, Indonesia) and sieved to obtain powder with a particle size of less than 0.5 mm to be used for the extractions.

Escin, Trolox and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Pty Ltd. (Singapore). Ethanol (analytical grade) was purchased from Merck Ltd. (Ho Chi Minh City, Vietnam).

2.2 Experimental design

The dried and ground PFR was extracted with 40% ethanol solution for 30, 60, 90, 120 and 150 mins to determine the most suitable extraction time. After determining the extraction time for the highest efficiency, the next experiment was performed with ethanol solutions with concentrations of 0% (distilled water), 20%, 40%, 60% and 80% to determine the most suitable ethanol concentration. The next experiment was to investigate the effect of temperature (30, 40, 50, 60 and 70^{o}C) to determine the best extraction temperature. Finally, the liquid-solid ratios of 5/1, 10/1, 20/1, 40/1 and 80/1 (mL/g) were used to investigate the effect of the solvent amount on the extraction efficiency.

2.3 Analytical methods

2.3.1 Determination of total saponin content

The content of total saponins in the material and extracts was determined by the spectrophotometric method (Nguyen, 2015). The vanillin-sulfuric acid reagent dissolves saponins and gives the reaction mixture a colour with an intensity that is directly proportional to the saponin concentration present in the sample. Based on the colour absorbance and the colour absorbance at 550 nm of the standard curve of the escin standard at different concentrations, the content of total saponins in the sample was calculated. Total saponin concentration was expressed as mg escin equivalent (mg EE)/g material.

2.3.2 Determination of total soluble solid content

The total soluble solid (TSS) content was determined by drying the samples to a constant weight at 105^{o}C (AOCS, 1998).

2.3.3 Determination of antioxidant activity

The antioxidant activity (AA) of the extract was determined by the DPPH free radical scavenging ability (2, 2-diphenyl-1-picrylhydrazyl) (Thaipong *et al.*, 2006). DPPH is a free radical with a deep purple colour having a peak spectral absorbance at 515 nm. When adding an antioxidant solution to the DPPH solution, the solution gradually loses its purple colour and turns yellow. The free radical scavenging ability of an antioxidative compound is directly proportional to the colour loss of the DPPH solution. Based on the colour absorbance of samples at 515 nm and the standard curve of Trolox, the AA of the extract is calculated. The AA of the extracts was expressed in mg Trolox equivalent (mg TE)/g of material.

2.4 Data analysis

All experiments were carried out in triplicates and the results are presented as the means ± standard deviations. The statistical significance was analyzed by one-way analysis of variance (ANOVA). The differences among mean values were compared using least significant difference (LSD) tests. Differences were considered to be statistically significant at $p < 0.05$.

3. Results and discussion

3.1 Effect of extraction time on the extraction efficiency

Figure 1A shows that the total saponins recovery and TSS content increased gradually over the extraction time. The extraction time of 120 mins gave the highest extraction efficiency of TSS and AA while the highest recovery of total saponins was obtained at 90 mins of extraction. This result suggests that the suitable time to achieve maximum saponins recovery is shorter than that of some other compounds from PFR. Previous studies have shown that PFR does not only contain saponins but also other compounds such as polyphenols, flavonoids or nutritional components such as polysaccharides and minerals. These components are more stable than saponins against the influence of temperature, oxygen and light. Therefore, when the extraction time is extended, the saponins are considerably degraded while

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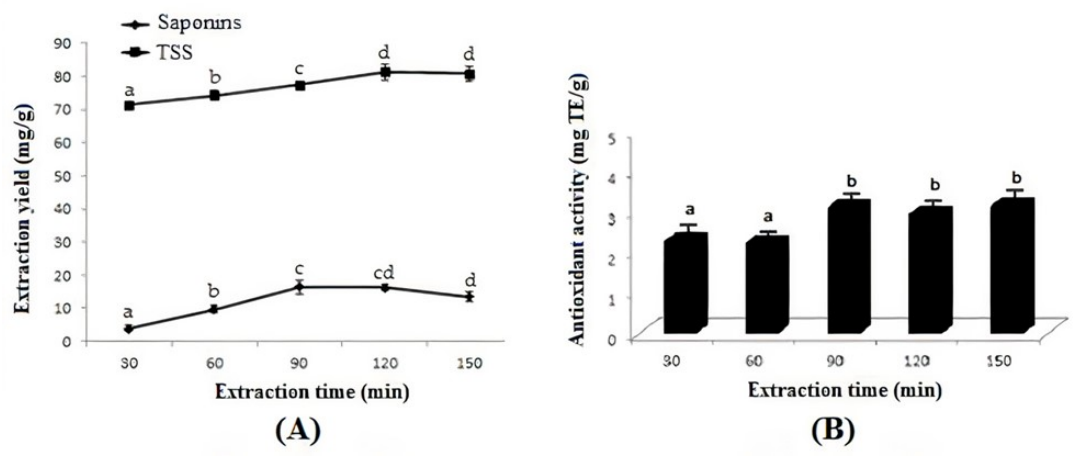

Figure 1. Effects of time on (A) extraction efficiency of total saponin and total soluble solid (TSS) and (B) the antioxidant activity (AA) of extracts. Bars with different notations are statistically significantly different among the values ($p < 0.05$).

other compounds continue to be diffused into the liquid phase, leading to an increase in the TSS content (Nguyen *et al.,* 2015; Liu *et al.*, 2016).

Figure 1B shows that the antioxidant activity (AA) of the extracts also depends on the extraction time. The highest AA was obtained at the extraction time of 90 mins and then fluctuated with negligible changes when the time was extended to 120 and 150 mins. Thus, the change in AA over time was similar to the change in the total saponins content of the extracts. This suggests that saponins had a substantial effect on the AA of the extract because although the TSS content still increased up to 120 mins of extraction, the AA of the extract obtained at this time did not significantly increase compared to that at 90 mins.

3.2 Effect of ethanol concentration

The dependence of saponins and total TSS yields on ethanol concentration is shown in Figure 2A. Total saponins extraction efficiency was strongly dependent on ethanol concentration while TSS extraction efficiency did not change significantly when the ethanol concentrations above exceeded 20%. The saponins yield increased as the ethanol concentration increased from 0 to 40% but the increase was not statistically significant when the concentration exceeded 40% (Figure 2A). This finding is in agreement with the results of previous studies on extracting saponins from Korean ginseng and other medicinal herbs (Kwon *et al.*, 2003; Nguyen, 2015). Results of those studies showed that the most suitable concentration for saponins extraction ranges from 40 to 65%. From the results of this study, 40% ethanol concentration was selected as the appropriate concentration for the extraction of saponins from PFR.

AA of the extracts obtained with different ethanol concentrations is shown in Figure 2B. The AA of the extracts obtained from ethanol concentrations of 40%, 60%, and 80% was significantly higher than that of the extract using 20% ethanol solvent and the extract using distilled water. As shown in Figure 2A, the TSS and total saponin recovered by ethanol solutions from 40-80% were significantly higher than those obtained by 20% ethanol solvent and distilled water. Therefore, the high content of saponins and other bioactive ingredients such as polyphenols, and flavonoids in the extracts using high ethanol concentrations led to a marked increase of the AA.

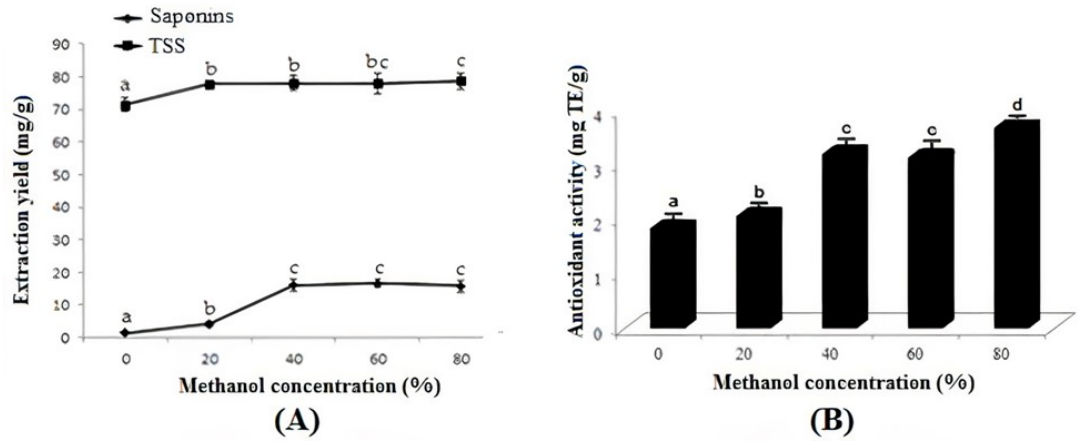

Figure 2. Effect of methanol concentration on (A) extraction efficiency of total saponin and total soluble solid (TSS) and (B) the antioxidant activity (AA) of extracts. Bars with different notations are statistically significantly different among the values ($p < 0.05$).

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3.3 Effect of extraction temperature

Figure 3A shows that the total saponin contents obtained at different temperatures are significantly different. The total saponin recovery efficiency was the lowest at 30°C and gradually increased to the highest value at the extraction temperature of 50°C. However, when the extraction temperature rose above 50°C, the extraction efficiency of saponins tended to be lower. This can be explained by the thermal instability of saponins. The diffusion of saponins from the solid material into the solvent gradually increased along with the increase of the temperature, leading to an improvement in the recovery efficiency, but at temperatures above 50°C, the degradation rate of saponins became faster than the diffusion rate thereby the recovery efficiency was reduced (Liu *et al.*, 2016).

As shown in Figure 3B, the highest AA was achieved at 50°C. At 60°C, the AA of the extract slightly improved but was not statistically significant compared to that at 50°C. For the extraction at a temperature of 70°C, a significant reduction in AA of the extract was observed compared to the extraction at 60°C. The decrease in AA of the extract at higher temperatures could be explained by a sharp decrease in saponin content in the extracts (Figure 3A). The reduction in AA along with the drop of total saponin content in the extracts while the TSS content remained stable at higher temperatures suggest that saponins predominantly contribute to the AA of the extracts from PFR.

3.4 Effect of liquid-solid ratio

Total saponin extraction efficiency increased sharply when the liquid-solid ratio increased from 5/1 to 20/1 (Figure 4A). This result is very consistent with the law of diffusion: the solute concentration gradient between the material and the solvent increases as the amount of solvent increases thus motivating the rate of diffusion of solutes from the material into the liquid phase. However, when the amount of solvent is too large, the diffusion distance from the solute molecule to the low concentration area of solvent would be increased. Therefore, the efficiency of saponins recovery did not increase significantly at the liquid-solid ratios of 40/1 and 80/1 compared to the ratio of 20/1.

Unlike the change in total saponin recovery efficiency, the AA of the extract continued to increase

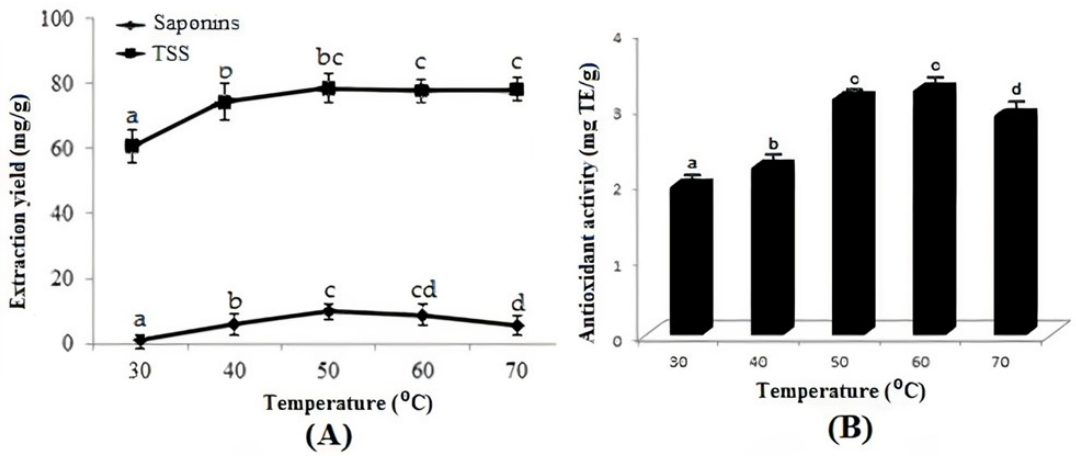

Figure 3. Effect of temperature on (A) extraction efficiency of total saponin and total soluble solid (TSS) and (B) the antioxidant activity (AA) of extracts. Bars with different notations are statistically significantly different among the values ($p < 0.05$).

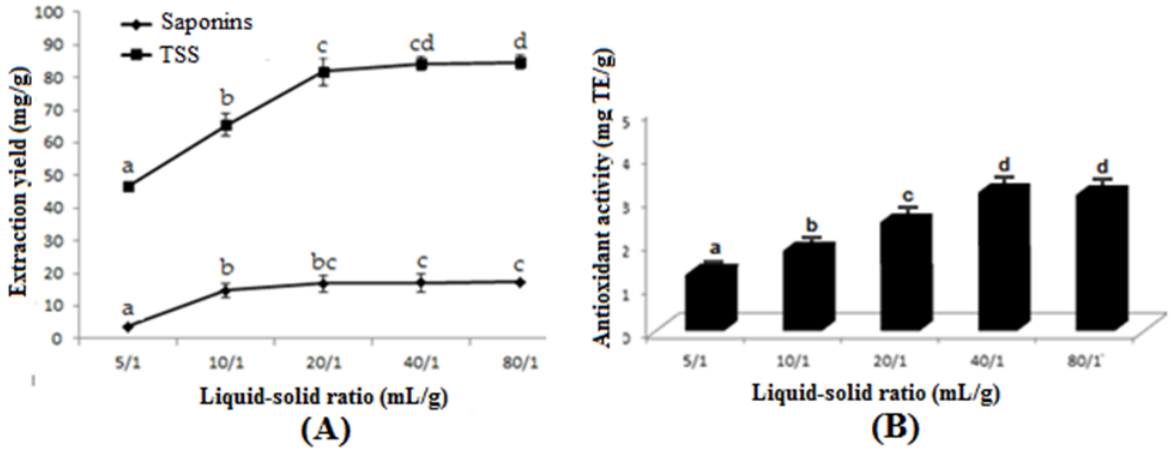

Figure 4. Effect of liquid-solid ratio on (A) extraction efficiency of total saponin and total soluble solid (TSS) and (B) the antioxidant activity (AA) of extracts. Bars with different notations are statistically significantly different among the values ($p < 0.05$).

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when the solvent-material ratio increased to 40/1(mL/g) (Figure 4B). As shown in Figure 3A, the improvement in the AA of the extract at this ratio may be caused by the increase in the extracted TSS content, not by the saponins. Previous studies have shown that not only saponins, but polyphenols also significantly contributed to the total soluble solid content of the extracts from *Polyscias fruticosa* roots (Bernard *et al.*, 1998; Vo *et al.*, 1998). Polyphenols are a group of bioactive compounds with very strong antioxidant activity, thus when these compounds were extracted more into the liquid phase at the ratio of 40/1 (mL/g), it would lead to the improvement in the AA of the PFR extract.

4. Conclusion

In conclusion, extraction yields of total saponin, TSS and AA from PFR were significantly affected by extraction time, temperature, ethanol concentration, and liquid-solid ratio. The highest extraction efficiency was obtained after 90 – 120 mins of the extraction process and ethanol with a concentration from 40 to 65% was found as the most suitable solvent for the extraction. As expected, the recovery of bioactive compounds from PFR improved along with the increase in the extraction temperature but that was limited when the temperature exceeded 50^{o}C due to the thermal sensitivity of saponins. Similarly, the utilization of a larger amount of solvent could accelerate the diffusion of bioactive ingredients into the liquid phase which resulted in higher recovering yields of total saponin, TSS and AA from PFR but the improvements were not significant at the liquid-solid ratios above 20/1 (mL/g). Therefore, the extraction process using 40% ethanol solution with the ratio of 20 mL of solvent/g of material at 50^{o}C for 90 mins is recommended for the recovery of saponins and antioxidant capacity from PFR.

Conflict of interest

The author declares no conflict of interest.

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