EFFECTS OF STORAGE CONDITIONS ON POLYPHENOL AND TRITERPENOID SAPONIN CONTENT AND THE ANTIOXIDANT CAPACITY OF ETHANOLIC EXTRACT FROM LEAVES OF *Polyscias fruticosa* (L.) Harms

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ABSTRACT

The objective of this study was to evaluate effects of temperatures (5 °C, 30 °C, and 60 °C) on the concentration of polyphenol and triterpenoid saponin and the antioxidant capacity of the ethanolic extract from the leaves of *Polyscias fruticosa* (L.) Harms during storage. Regression analysis revealed that the antioxidant capacity highly correlated with the content of polyphenol and triterpenoid saponin. After 30 days, the extract stored at 5 °C showed the decreases of total triterpenoid saponin, total polyphenol, DPPH free radical scavenging, and ferric reducing power were 9.55%, 19.81%, 4.18%, and 34.6%, respectively. These values were lower than those after storage at 30 °C and 60 °C. Thus, storage of the extract at low temperature (5 °C) will restrict losses of the extract's bioactivity and improve the quality of final products.

Keywords: Polyscias fruticosa (L.) Harms, total triterpenoid saponin content, total polyphenol content, antioxidant capacity, storage temperature.

1. INTRODUCTION

Polyscias fruticosa (L.) Harms belongs to the family Araliaceae and widely distributes in Vietnam as well as many countries of southeast Asian. In Vietnam, the leaves of this plant are used as a tonic agent for the treatment of ischemia and inflammation [1, 2]. They are also eaten as a salad [1]. In the *Polyscias fruticosa* leaves, the two important groups of biological compounds are polyphenol and triterpenoid saponin [2, 3]. Some previous studies showed that the leaf extract of *Polyscias fruticosa* contains some bioactive components (i.e., alkaloids, steroid, saponins, flavonoids, tannins, and vitamins) [1, 3]. These components showed anti-inflammatory, analgesic, and analgesic activities [2, 4, 5].

In recent years, many products of *Polyscias fruticosa* have been developed and considered as an alternative medicine [1, 4]. The stability of the bioactive compounds of *Polyscias fruticosa* product during processing and storage is a continuous challenge. According to Nguyen and Hoang [6], the temperature and time of processing have a strong effect on the stability of the bioactive compounds of the *Polyscias fruticosa* extract. The total flavonoid, saponin, and phenolic content significantly decreased with increased temperature and storage time. These authors also reported that the optimum storage temperature and time for *Polyscias fruticosa* herb drink was 4 °C and 6 weeks. Similar results were also reported

for *Polyscias fruticosa* dried leaf tea [7]. However, there exists no research on the effect of storage conditions on the antioxidant activity of the leaf extracts of *Polyscias fruticosa*.

Thus, the objective of this study was to investigate the effect of storage temperature (5 °C, 30 °C, and 60 °C) on total triterpenoid saponin, total polyphenol content, and antioxidant activity.

2. MATERIALS AND METHODS

2.1. Materials

The leaves of *Polyscias fruticosa* (L.) Harms were collected in Thanh Hoa District, Long An Province, Vietnam. Then, they were naturally dried, ground, milled, sieved through a 0.3 mm sieve and stored in zip bags. The obtained powder had moisture content of 4.2 ± 0.2%, protein of 15.6 ± 0.7%, lipid of 7.3 ± 0.3% and carbohydrate of 49.1 ± 1.2%.

Chemicals used were as follows: For the determination of total polyphenol content (TPC): Folin-Ciocalteu's phenol reagent (Merck, 2 N), gallic acid (Sigma, 98%); For the determination of total triterpenoid saponin: Oleanolic acid (Vietnam, 90%), vanilin (Sigma, 99%), perchloric acid (Merck, 70%), acetic acid (Merck, 95%); For the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay: 1,1-Diphenyl-2-picrylhydrazyl (Sigma, 97%), trolox (Sigma, 97%), ethanol (Merck, 95%); For the Ferric reducing powder assay: Trichloroacetic acid (TCA) (Merck, 99%), potassium hexacyanoferrate (III) (Sigma, 99.98%).

2.2. Methods

2.2.1. Preparation of the ethanolic extract

The sieved *Polyscias fruticosa* powder (50 grams) was extracted with 1000 mL ethanol 95% for 60 minutes at room temperature. Then, the mixture was centrifuged at 5000 rpm for 15 minutes. The solution obtained after extraction was concentrated in a rotary evaporator at the temperature of 60 °C with the pressure of 500 mmHg. After preparation, the extract was stored in the dark at temperatures of 5 °C, 30 °C and 60 °C for 30 days. After each day of storage, samples were analyzed for their total triterpenoid saponin content, total polyphenol content and antioxidant activity (including DPPH free radical scavenging and ferric reducing power).

2.2.2. Determination of total polyphenol content

The total polyphenol content was determined following Vernon *et al.* (1999) with minor modifications [5]. Briefly, the extract (0.5 mL) was taken into the test tube and 2.5 mL Folin- Ciocalteu's reagent was added to it. The mixture was then mixed and incubated for 5 minutes before adding 2.0 mL of Na_2CO_3 7.5%. After 1-hour incubation at the ambient temperature, the absorbance was taken at 765 nm against a blank by using UV-spectrophotometer/NIR (Shimazu, UV-2600, Japan). The total polyphenol content was equivalent to the standard gallic acid content in 1 mL extract (μgGAE/mL).

2.2.3. Determination of total triterpenoid saponin content

The total triterpenoid saponin content was determined according to Gao's method. A mixture of vanillin-acetic acid (5% w/v, 2 mL) was mixed with 1.2 ml perchloric acid and kept at 60 °C for 20 minutes. After that, 0.2 mL of the extracts was added to the mixture and incubated at 70 °C for 15 minutes before adding 5 mL ethyl acetate. The solution was let to stand for 1 minute in dark place. The absorbance was measured at 548 nm using UV-

spectrophotometer/NIR (Shimazu, UV-2600, Japan) and oleanolic acid was used for comparison. The total triterpenoid saponin content was expressed in mg/mL based on the calibration curve with the standard oleanolic acid [8].

2.2.4. Determination of antioxidant activity

2.2.4.1. Ferric reducing power assay

Ferric reducing antioxidant power (FRAP) assay measured the total reducing power of electron donation substances following Oyaizu *et al.* [9]. 1.0 mL sample was mixed with 2.5 mL of phosphate buffer pH 6.6 and 0.5 mL 1% of $K_3(Fe[CN])_6$ solution. The mixture was then incubated at 50°C for 20 minutes and let to cool to room temperature. A 2.5 mL 10% of TCA was added and thoroughly mixed, after that, 2.5 mL of solution was withdrawn and added 2.5 mL water followed by 0.5 mL of $FeCl_3$ solution. The absorbance of the resulting Prussian blue solution at 700 nm was measured. Ferric reducing power was expressed based on the calibration curve with the Trolox standard and the unit was mg Fe^{3+} reduced by 1 mL of extract (mgFe^{3+}/mL).

2.2.4.2. DPPH free radical scavenging assay

DPPH free radical scavenging was determined by the method of Fu *et al.* with some modification [10]. 1 mL of diluted extract at a suitable concentration was mixed with 5mL DPPH 30 mM, shaken and incubated for 30 minutes. The absorbance of the resulting solution at 517 nm was measured. DPPH free radical scavenging was equivalent mg of Trolox in 1 mL extract (mg Trolox/mL).

2.3. Statistical analysis

All the experiment values were statistically analyzed by Stagraphic centrution XV (Statsoft Inc., USA). The results were expressed as mean ± SD and a 5% significance was used in all cases.

3. RESULTS AND DISCUSSION

3.1. Changes of total polyphenol content and correlation between total polyphenol content and antioxidant activity

Figure 1 shows the result regarding the change of total polyphenol content and its antioxidant capacity at different temperatures for 30 days. As shown in Figure 1, the total polyphenol content of the extract storage at 5 ºC slightly decreased from 70.15 to 67.81 μgGAE/mL at 14 days, whereas after 14 days storage at 30 ºC and 60 ºC, the total phenolic contents reduced rapidly to 57.63 μgGAE/mL and 52.71 μgGAE/ mL, respectively. However, the polyphenol content of these extracts did not change much in all temperature conditions and stably remained at the end of storage ($p < 0.05$). This result was similar to Zam's report about the change in polyphenol content in pomegranate fruit extract at different storage temperatures for 14 days [11]. The author showed that temperature and storage time affected the total polyphenol content. Specifically, the change of polyphenol content of the samples stored at low temperature was significantly lower than those stored at 60 ºC and at room temperature.

This observation could be explained by the fact that polyphenols are sensitive antioxidants, so during storage, some polyphenols may be involved in oxidase reaction to protect the extract from the attack of oxidizing agent available in the sample. In 14 days, when the oxygen content was high, polyphenols involved in the reaction led to a significant decrease in the total polyphenol content. Besides, the study by James *et al*. reported that the

rate of reaction was proportional to temperature [12]. The high temperature (60 °C) will promote the reaction to be faster and more intense than that at the low temperature (5 °C).

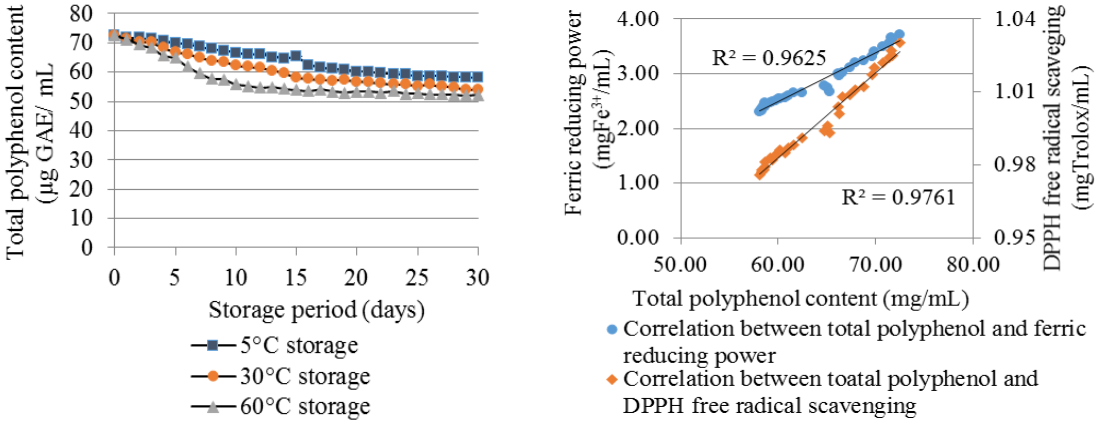

Figure 1. Changes of total polyphenol content during storage at different temperatures (a) and correlation between total polyphenol and antioxidant activity at 5 ºC storage (b)

Figure 2 also showed that FRAP and DPPH decreased with increasing temperature and storage time. FRAP and DPPH at 5 °C decreased more than 31.45% (3.71 mgFe^{3+}/mL to 2.31 mgFe^{3+}/mL) and 3.41% (1.025 to 0.99 mgFe^{3+}/mL) during 30 days of storage. In contrast, the decrease for 30 °C and 60 °C storage samples were 34.46% and 49.07%, respectively, which were significantly higher than those stored at 5 °C. Similarly, DPPH free radical scavenging also showed a decrease as the temperature and storage time increased. The phenolic group significantly showed the antioxidant activity proved to be stronger than vitamin C, E and carotenoids [10 - 17].

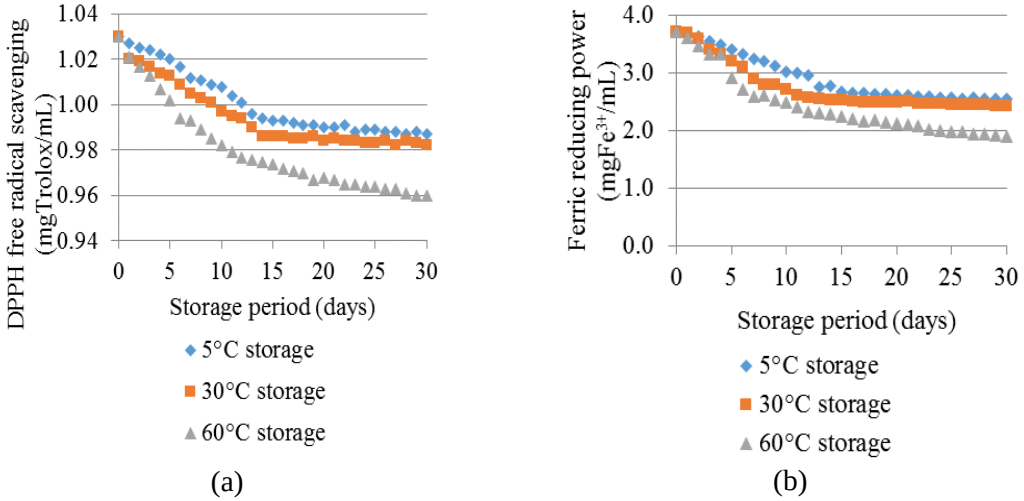

Figure 2. The change of DPPH free radical scavenging (a) and ferric reducing power (b) in 30 days storage at different temperatures.

The line chart (Figure 1b) showed that DPPH and FRAP followed the change of polyphenol content. The results illustrated the antioxidant capacity by the DPPH assay ($y = 0.0457x - 1.34543$, $R^2 = 0.9761$) was more strongly positively correlated with total polyphenol content when compared to the ferric reducing power ($y = 0.0897x - 2.88943$, $R^2 = 0.9625$). Our results suggested that the antioxidant capacity and the total phenolic content were better

reflected by DPPH assay than FRAP assay. The correlation between the total polyphenol and the antioxidant activity was analyzed in several studies, such as Bambang *et al.*'s, Kettawan *et al.*'s reports on seaweeds also showed that the antioxidant activity had also increased following by high levels of polyphenol [15, 16]. Thus, these results proved that in order to retain the highest total polyphenol content and the highest antioxidant activity, the extract should be kept at as low a temperature as possible.

3.2. Changes of total triterpenoid saponin content and its correlation with the antioxidant activity

Figure 3 illustrated the reduction of triterpenoid saponin throughout storage at all temperatures. Specifically, the triterpenoid saponin content of the 5 °C storage samples showed a slight decrease by 9.55% (1050.12 mg/mL to 949.79 mg/mL) after 30 days, while those of the 30 °C and 60 °C storage samples decreased sharply by 29.65% (1050.12 mg/mL to 738.73 mg/mL) and 57.82% (1050.12 mg/mL to 442.94 mg/mL), respectively. This result was similar to the Du *et al.*'s study about American Ginseng in 12 weeks of storage at 5 °C, 20 °C, and 30 °C indicating that the total triterpenoid saponin at all temperatures tended to decrease [17].

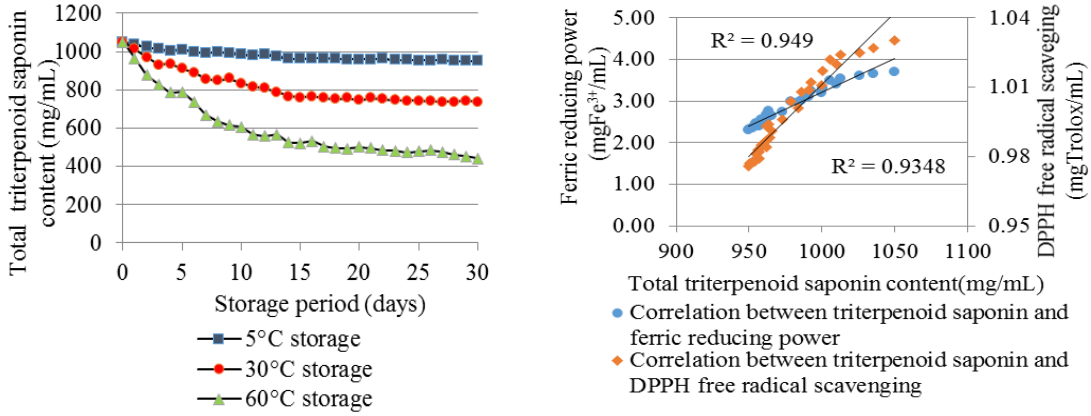

Figure 3. Changes in the total triterpenoid saponin content during storage at different temperatures (a) and correlation between the total triterpenoid saponin and antioxidant activity at 5 °C storage (b)

This result also illustrated that the sample's decreasing rate stored at higher temperatures was higher than that of the low temperature. From 19 days to 30 days of storage, triterpenoid saponin at 5 °C and 30 °C did not change much, whereas at 60°C it decreased sharply. Our finding is similar with Du *et al.*, who reported that when the temperature was over 30 °C, the total triterpenoid saponin in American ginseng decreased by three- and two-fold, compared to those in 14-day storage at 5 °C and 20 °C, respectively [17].

The line chart (Figure 3b) showed that DPPH and FRAP followed the change of triterpenoid saponin content at different temperature conditions. As shown in this study, the extract stored at 5 °C for 30 days had higher DPPH and FRAP value than those stored at 30 °C and 60 °C. This could be explained by the fact that the high temperature (30 °C and 60 °C) could increase the reaction of triterpenoid saponin with the presence of oxygen. Besides, the regression analysis showed that the triterpenoid saponin content was positively correlated with DPPH and FRAP ($R^2 > 0.93$) (Figure 3b). The results are similar with Bi *et al.*, who documented a positive correlation between the saponin content and the antioxidant activity [18]. Therefore,

the high storage temperatures were proven to cause undesired reactions that affect the triterpenoid saponin and total polyphenol content - two factors strongly influence on the antioxidant activity of the extract.

4. CONCLUSIONS

This present study clearly showed the strong correlations between the total polyphenol, total triterpenoid saponin with the antioxidant activity of the *Polyscias fruticosa* (L.) Harms extract. The low temperature was chosen to retain most of the total polyphenol, triterpenoid saponin and bioactivity of the extract.

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TÓM TẮT

ẢNH HƯỞNG CỦA ĐIỀU KIỆN BẢO QUẢN LÊN HÀM LƯỢNG POLYPHENOL VÀ SAPONIN TRITERPENOID VÀ KHẢ NĂNG KHÁNG OXY HÓA CỦA DỊCH CHIẾT ETHANOL TỪ LÁ ĐINH LĂNG *Polyscias fruticosa* (L.) Harms

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Nghiên cứu này đánh giá ảnh hưởng của nhiệt độ (5 ^oC, 30 ^oC và 60 ^oC) lên hàm lượng polyphenol tổng, hàm lượng triterpenoid saponin tổng và khả năng kháng oxy hóa của dịch chiết ethanol từ lá đinh lăng *Polyscias fruticosa* (L.) Harms trong suốt quá trình bảo quản. Phân tích hồi quy cho thấy khả năng kháng oxy hóa có sự tương quan cao với hàm lượng polyphenol và triterpenoid saponin. Sau 30 ngày bảo quản ở 5 °C, mức độ giảm của hàm lượng triterpenoid saponin tổng, polyphenol tổng, khả năng bắt gốc tự do DPPH và năng lực khử sắt lần lượt là 9,55%, 19,81%, 4,18% và 34,6%. Những giá trị đó thấp hơn so với điều kiện bảo quản ở nhiệt độ 30 ^oC và 60 ^oC. Vì thế, bảo quản dịch chiết ở nhiệt độ thấp sẽ giúp hạn chế sự thất thoát của các hoạt tính sinh học có trong dịch chiết và cải thiện được chất lượng của sản phẩm cuối cùng.

Từ khóa: *Polyscias fruticosa* (L.) Harms, hàm lượng triterpenoid saponin tổng, hàm lượng polyphenol tổng, khả năng kháng oxy hóa, nhiệt độ bảo quản.