

EFFECT OF EXTRACTION CONDITIONS ON TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF ROSELLE CALYCES (*HIBISCUS SABDARIFFA* L.) EXTRACT

ẢNH HƯỞNG CỦA ĐIỀU KIỆN TRÍCH LY ĐẾN HÀM LƯỢNG POLYPHENOL TỔNG SỐ VÀ KHẢ NĂNG KHÁNG OXY HÓA CỦA DỊCH TRÍCH ĐÀI HOA BỤY GIẤM (*HIBISCUS SABDARIFFA* L.)

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ABSTRACT

The calyx of the roselle plant has long been recognized as a source of antioxidants. The objective of this study was to investigate the effect of extraction conditions including extraction time; extraction temperature and ratio of water to material weight on total solid content, total polyphenol content and DPPH radical scavenging activity of roselle calyces extract solution. The results showed that total solid content, total polyphenol content and DPPH radical activity of extraction solution of roselle calyces significantly depended on extraction conditions. Moreover, the DPPH radical scavenging activity showed a positive relationship with total polyphenol content. The roselle calyces extracted at 90⁰C, for 30 mins and at the ratio of water/roselle calyces of 10/1 were the most suitable conditions for extraction of total polyphenol content and antioxidant components.

Keywords: Biosorption; Cr (VI); Modified chitosan beads; *Saccharomyces cerevisiae*; Histidine.

TÓM TẮT

Đài hoa bụp giấm có chứa nhiều hợp chất có hoạt tính sinh học quý, tuy nhiên chúng có thể bị biến đổi trong quá trình trích ly làm giảm giá trị dinh dưỡng. Nghiên cứu này tiến hành khảo sát ảnh hưởng của nhiệt độ nước trích ly, tỉ lệ nước, thời gian trích đến hàm lượng chất khô hòa tan, hàm lượng polyphenol tổng số, khả năng kháng oxy hóa thông qua khả năng dập tắt gốc tự do 1,1-diphenyl-2-picryl hydrazyl của đài hoa bụp giấm khô. Kết quả cho thấy, hiệu suất trích ly, hàm lượng polyphenol tổng số và khả năng kháng oxy hóa của dịch trích ly từ đài hoa bụp giấm phụ thuộc vào điều kiện trích ly gồm nhiệt độ nước trích ly, thời gian trích ly và tỷ lệ nước/nguyên liệu. Điều kiện trích ly thích hợp trong nghiên cứu này là thời gian trích ly 30 phút, nhiệt độ trích ly là 90⁰C, tỷ lệ nước/nguyên liệu là 10/1; ở điều kiện này, dịch trích ly có hàm lượng polyphenol tổng số và khả năng kháng oxy hóa của dịch trích là cao nhất.

Từ khóa: Calyces of Roselle; DPPH radical scavenging; total polyphenol content.

1. INTRODUCTION

Roselle calyces and petals have well known about valuable pharmacological

activities such as antioxidant and antimutagenicity activities. Recently, many reseaches have been paid attention to

polyphenol compounds presenting in the medicinal plants. Several authors reported that polyphenols from plants possess several bioactivities such as antioxidant and anticancer activities [1]. The method for extraction compounds of polyphenol and antioxidant activity were influenced by extraction conditions such as extraction time, extraction solvent [2]; pH and ratio of the solvent [3,4]. The objective of this study was to investigate the effects of extraction conditions on antioxidant activity of roselle calyces collected in Dak Lak province.

2. MATERIALS AND METHODS

2.1 Plant materials

Fresh roselle calyces (*Hibiscus sabdariffa*) were purchased from Curbua town, Buon Ma Thuot city, Dak Lak province. The chosen calyces were dried at 80 °C to obtain the moisture of 8% and stored in black PE bag then was kept in minus 30 °C or used immediately.

2.2 Chemical reagents

Chemical reagents including DPPH (2,2-diphenyl-2-picryl hydrazyl), and gallic acid were from Sigma-Aldrich (St Louis, MO, USA), the solvent and other chemicals were analytically obtained.

2.3 Analytical method

a. Sample preparation

3g of dried roselle calyces was weighed by an analytical balance then put in a 250ml conical flask. The extraction was performed in a water bath with standard conditions about temperature, time and ratio of water to roselle calyces. The filtered supernatant was adjusted up to 50ml in a volumetric flask. The extract was stored in cold dry place and avoided direct lighting.

b. Determination of antioxidant activity by DPPH radical scavenging using modified method of Nguyen and Eun [5].

c. Determination of total polyphenol content by the Folin-Ciocalteu method [6].

2.4. Statistical analysis

Data were reported with standard deviation (SD) of triplicate determination. Statistical calculations by Statgraphic Centurion XVI.II. Values of $p < 5\%$ were considered as significant difference.

3. RESULTS AND DISCUSSION

3.1 Effect of extraction temperature

Samples were extracted with the extraction temperature of 70, 80, 90 and 100 °C at a ratio of water/dried roselle of 10/1 for 20 min. Total soluble solid, total polyphenol contents and antioxidant activity were presented in Figure 1, 2 and 3, respectively.

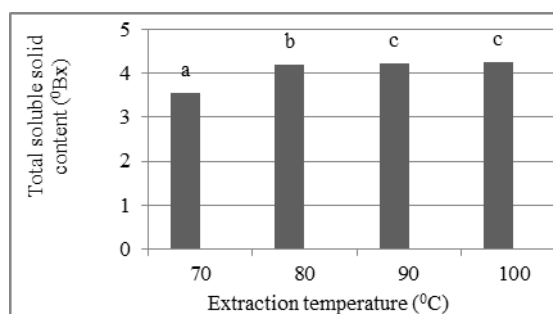


Figure 1. Effect of temperature of extraction water on total soluble solid content. a, b, c above the bar indicate a significant difference at $p < 5\%$

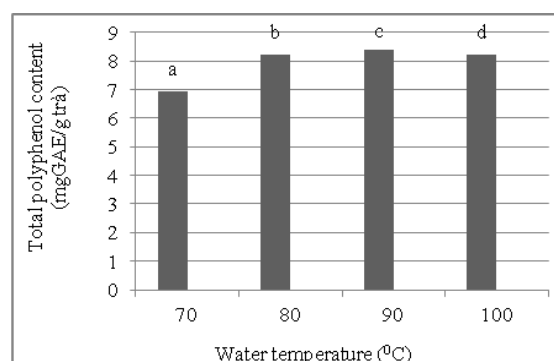


Figure 2. Effect of water extraction temperature on total polyphenol content. a, b, c, d above the bar indicate a significant difference at $p < 5\%$

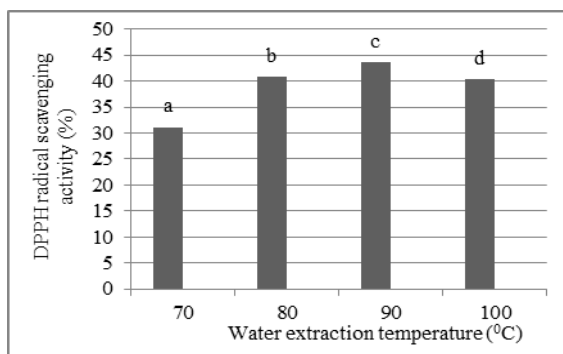


Figure 3. Effect of water extraction temperature on DPPH radical scavenging activity. a, b, c, d above the bar indicate a significant difference at $p < 5\%$

Figure 1 shows that the total soluble solid contents of the extract depended on extraction temperature. An increase in extraction temperature of water from 70°C to 100°C was accompanied by an increase in soluble solid contents from 3.567⁰Bx to 4.267⁰Bx. However, soluble solid contents of sample extracted at 90°C and 100°C, were not significantly increase ($p < 0.05$).

Total polyphenol contents were different in various extraction temperatures which were shown in Figure 2. The extraction of phenolic contents were increased (from 6.944mg/g to 8.38 mg/g) when extraction temperature increased from 70°C to 90°C. However, it should be noted that when temperature of water was about 100 °C, total polyphenols tended to decrease by 8.233mg/g. This observation could be explained by the invading of water to the samples which was convenient by rising temperature led to improve efficiency of extraction. Nevertheless, increasing to high temperature may promote oxidation reaction of polyphenols or reaction between calyces composition and polyphenol compounds. This phenomenon was in accordance with previous studies [8].

The effect of extraction temperature on DPPH radical scavenging activity was showed in Figure 3. The temperature increased from 70°C to 90°C resulted in an increasing in free

radical scavenging capacity from 31.103% to 43.565%. However, free radical scavenging activity decreased when temperature rose to 100°C. It was probably because the growth of temperature from 80°C to 90°C enhanced the extraction of antioxidant compounds, but if the temperature was too high (about 100°C), antioxidant substances could be reduced.

In general, the optimized extraction temperature to extract antioxidant compounds in dried reselle calyces was 90°C. At this temperature, the economic efficiency and amount of antioxidants in roselle calyces were the highest.

3.2 Effect of water/calyces ratio

The extraction was performed on different ratios of water to roselle calyces (8/1, 10/1, 12/1, 14/1) at 90°C for 20 min. Total soluble solid content, total polyphenols and antioxidant activity of extract were illustrated in figures 4, 5, 6 respectively.

Figure 4 shows the ratio relation between water and dry solid concentration. An increase in water to botanical materil ratio from 8/1 to 12/1 was accompanied by an increment of dried weight from 1.367 to 2⁰Bx. However, there was no significant difference existed when increasing the ratio after 14/1. Therefore, soluble concentration of the extract depended on water/roselle calyces ratio.

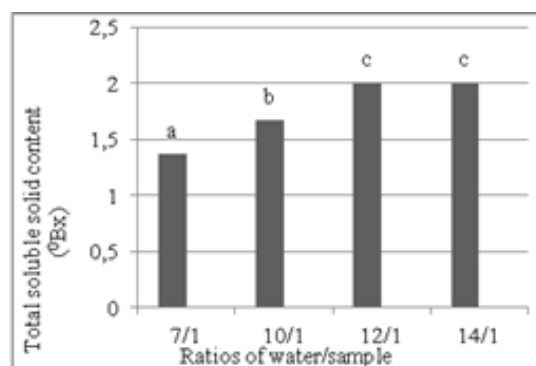


Figure 4. Effect of the ratio of water/sample on total soluble solid content. a, b, c above the bar indicate a significant difference at $p < 5\%$

The ratio of water/sample increase from 8/1 to 10/1 was associated with the increase of polyphenol content from 7.588 to 10.12mg GAE/f (Figure 5). Nevertheless, when continued increasing the ratios of water/roselle calyces, the results were not significantly.

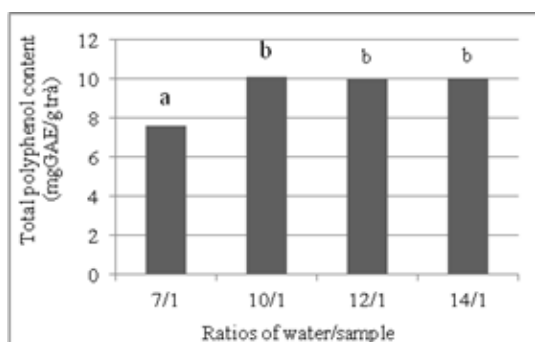


Figure 5. Effect of the ratio of water/sample on total polyphenol contents. a, b, c above the bar indicate a significant difference at $p < 5\%$

This outcome was similar to antioxidant activity of roselle calyces' extract. The Figure 6 displayed the most suitable water/roselle calyces ratio for extraction of antioxidant capacity compound was 10/1. Although the total soluble solid content at this ratio was smaller than that at the ratio of 12/1; however, the antioxidant activity of the extract was higher at the ratio of 10/1. The results were accordance with the report of Vu Hong Son and Ha Duyen Tu [6].

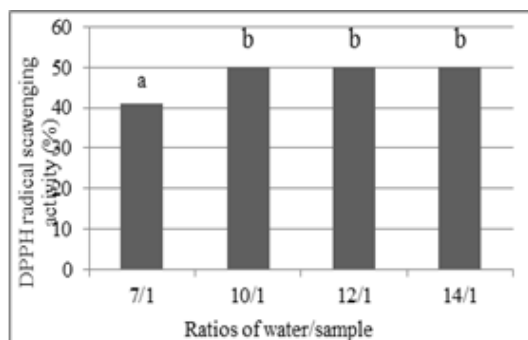


Figure 6. Effect of the ratio of water/sample on DPPH radical scavenging activity. a, b above the bar indicate a significant difference at $p < 5\%$

3.3 Effect of extraction time

Roselle calyces were extracted for 10, 20, 30 and 40 min with the ratio of water to sample of 10/1 and temperature extraction of water of 90°C . Total soluble solid, total polyphenol contents and antioxidant capacity of extract were presented on figure 7, 8 and 9, respectively.

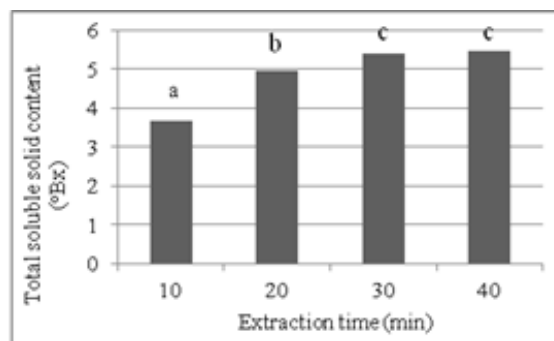


Figure 7. Effect of extraction time on total soluble solid content. a, b, c above the bar indicate a significant difference at $p < 5\%$

Figure 7 shows the result that soluble solid content of the extracts at different extraction time were significant different. The highest solid concentration was recorded in sample extracted for 30 min of 5.3°Bx . When increasing extraction time from 10 to 30 min, solid concentration rose from 3.6°Bx to 5.3°Bx . Nonetheless, there was no significant difference after 40 min of extraction.

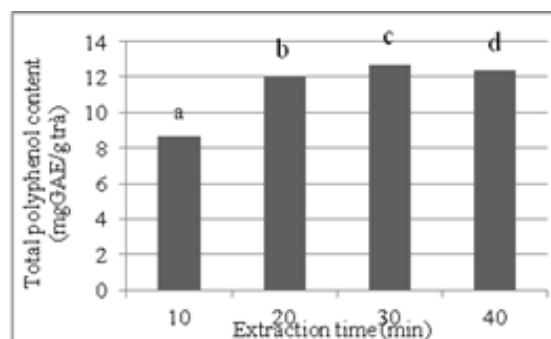


Figure 8. Effect of extraction time on total polyphenol contents. a, b, c, d above the bar indicate a significant difference at $p < 5\%$

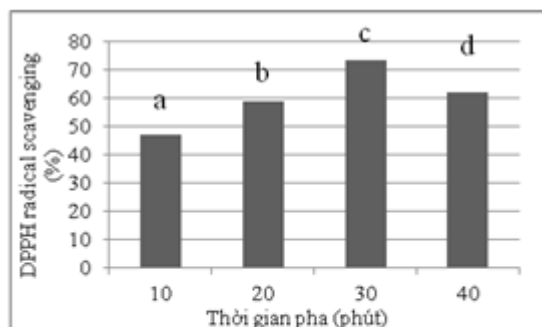


Figure 9. Effect of extraction time on DPPH radical scavenging activity
a, b, c, d above the bar indicate a significant difference at $p < 5\%$

Figure 8 displays total polyphenol in extract increased from 8.66 mg/g to 12.70 mg/g from 10 to 30 min of extraction time. However, total polyphenol tended to decrease when extraction time was over 30 min.

DPPH radical scavenging ability of sample went up from 46.9% to 73.3% whereas extraction time rose from 10 to 30

min. Nevertheless, the figure 9 shows that the percentage of DPPH radical scavenging ability diminished to 62.1% when extraction time increased to 40 min. The conducted regression analysis shows that there is a strong positive correlation between antioxidant ability and total polyphenol content. This result was also published by some authors previously [3]. Therefore, the suitable extraction time for antioxidant substances of roselle calyces was 30 min.

4. CONCLUSIONS

In this study, all the extraction parameters (extraction time, extraction temperature, and water/sample ratio) showed significant effect on antioxidant ability of roselle calyces extract. The suitable conditions for extraction were of extraction temperature of 90⁰C, water/roselle calyces ratio of 10/1 and extraction time of 30 min.

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