Impact of extract conditions on total phenolic compounds and antioxidant activities of Phu Quoc sim fruits (*rhodomyrtus tomentosa* (ait.) hassk.)

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Abstract

Rhodomyrtus tomentosa is an evergreen shrub that originates from Southeast Asia and grows in abundance with rose-pink flowers and dark-purple edible bell-shaped fruits. In this study, optimal extraction conditions for achieving high contents and antioxidant activities of the total phenolic compound (TPC) from Phu Quoc sim fruits were investigated. It was found that the total phenolic content achieved was upon 179.055mg gallic acid equivalent (GAE)/g dry weight extract (DWE) and the DPPH scavenging activity was 65.112% at concentration of 100µg/ml. The optimal extraction conditions were identified with 80% ethanol, with solid-to-solvent ratio of 1/4 (w/v), and 4 hours of extraction at the temperature of 60°C. Furthermore, the antioxidant activity of ethanol extract was found due to scavenging DPPH (IC_{50}=93.713µg/ml) and ABTS (IC_{50}=83.512µg/ml) radicals. Accordingly, Phu Quoc sim fruits was suggested a potential source of bioactive polyphenols with high antioxidant activities.

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Keywords
Sim, *Rhodomyrtus tomentosa*, phenolic, DPPH, antioxidant

1 Introduction

Phenolic compounds constitute one of most numerous and widely distributed groups of phytochemicals in the plant kingdom. More than 8,000 distinct structures are reportedly known and identified. Polyphenols are products of secondary metabolism and can vary from simple phenolic acids to highly polymerized molecules such as tannins[4,23] Phenolic compounds have been considered to have high antioxidant ability and free radical scavenging capacity, with the mechanism of inhibiting the enzymes responsible for ROS production and reducing highly oxidized ROS[15,20]. Therefore, phenolic compounds have attracted increasing attention as potential agents for preventing and treating many oxidative stress-related diseases, such as cardiovascular diseases, cancer, ageing, diabetes mellitus and neurodegenerative diseases.

Rhodomyrtus tomentosa, locally known as Sim, is a flowering plant belonging to the Myrtaceae family, which is native to southern and southeastern Asia. Parts of this plant have been used in traditional Vietnamese, Chinese and Malay medicine for a long time. In particular, the fruits have been used to treat diarrhea, dysentery, gynaecopathy, stomachache, and for wound healing[5,11]. The chemical constituents of *R. tomentensa* have been reported to include triterpenes, steroids, and especially phenolic compounds. Although phenolic compounds from *R. tomentensa* have been reported recently, the further studies on their biological activities are still limited. Thus, the main purpose of this project is to evaluate the optimal extraction conditions for achieving high content and antioxidant activities of the total phenolic compound from Phu Quoc sim fruits (*R. tomentosa*).

2 Materials and methods

2.1 Fruits powder

The mature fruits of *R. tomentosa* (Ait.) Hassk were purchased from Duong Dong town, Phu Quoc in March, 2018. Fruits were washed with sterilized water, dried, then crushed with grinder and sealed in dark color bottles at -20°C until further analyses.

2.2 Effects of extraction parameters

Various parameters examined in this study included solvent concentration (60%, 80%, 100%), solid to solvent ratio

(SSD) (1: 1, 1: 2, 1: 4, 1: 8), extraction time (1h, 4h, 12h, 24h) and extraction temperature (t°p, 40°C, 60°C). These parameters are indicated to affect extraction efficiency and phytochemical contents[6].

For examination of solvent degree, 1 gram of Sim fruits powder was soaked in various ethanol degree with the initial fixed parameters. In detail, the initial fixed parameters were 1:4 solid to solvent ratio, room temperature, and 24 hours of extraction. The subsequent experiments inherited the previous results for further investigations. The extracted solutions were evaporated to achieve the extracts for total phenolic compounds and antioxidant assays.

2.3 Phytochemical analysis

2.3.1 Total phenolic compounds (TPC) analysis

The total phenolic compounds in ethanol extract were determined using Folin-Cocialteau method[21]. Briefly, 500µl of Folin-Cocialteau 10% solution (w/v) was mixed with 100µl extracted solution (1mg/ml),and added 400µl Na_2CO_3 7.5% (w/v) after reacting for 5 minutes. The reaction mixture was kept in dark at room temperature for 60 minutes, then the absorption at 750nm was measured. The total phenolic content was calculated based on the standard curve of gallic acid. The formula is as follows:

$$\text{TPC (mgGAE/g)} = Cx \times 10^{-3} \times V \times \frac{100}{m \times (100-h)}$$

In particular, TPC is the total amount of polyphenols present in the sample which expressed as mg gallic acid per 1g dry weight extract, Cx is µg of gallic acid per 1ml extracted solution inferred from the standard curve, 10^{-3} is the factor of transitions from µg to mg, V is the volume of the sample (ml), m is the mass of the extract (g), and h is the moisture of extract (%).

2.3.2 DPPH assay

DPPH (2,2-diphenyl-1-picryl-hydrazyl) is a method to determine antioxidant capacity. It was developed by Blois[2]. The assay is based on the change of DPPH solution color when odd electron in DPPH free radical is reduced by pairing off with a hydrogen radical from antioxidants to become a stable molecule[8]. In this study, 100µl of extracted solution reacted with 100µl DPPH (3mM) in dark room for 30 minutes. The mixtures were measured at 490nm for determining the absorption. Vitamin C was used as positive control. The scavenging ability was calculated using the following formula:

$$\text{DPPH scavenging ability (\%)} = (1 - \frac{ODs}{ODb}) \times 100$$

Here, ODs is the value for sample absorption and ODb is the value for blank absorption.

2.3.3 ABTS assay

In short, the ABTS solution was prepared by mixing 2.45mM postassium persulfate with 7mM ABTS stock solution (1/1, v/v) and diluted to the absorption of 0.7 ± 0.02 after 6-16 hours at 734nm. The assay was conducted with 0.2ml extract solution and 1.8ml ABTS solution in 6 minutes. The absorbance was then measured at the wavelength of 734nm. Scavenging effect can be determined by following equation:

$$\text{ABTS scavenging ability (\%)} = (1 - \frac{ODs}{ODb}) \times 100$$

Here, ODs is the value for sample absorption and ODb is the value for blank absorption.

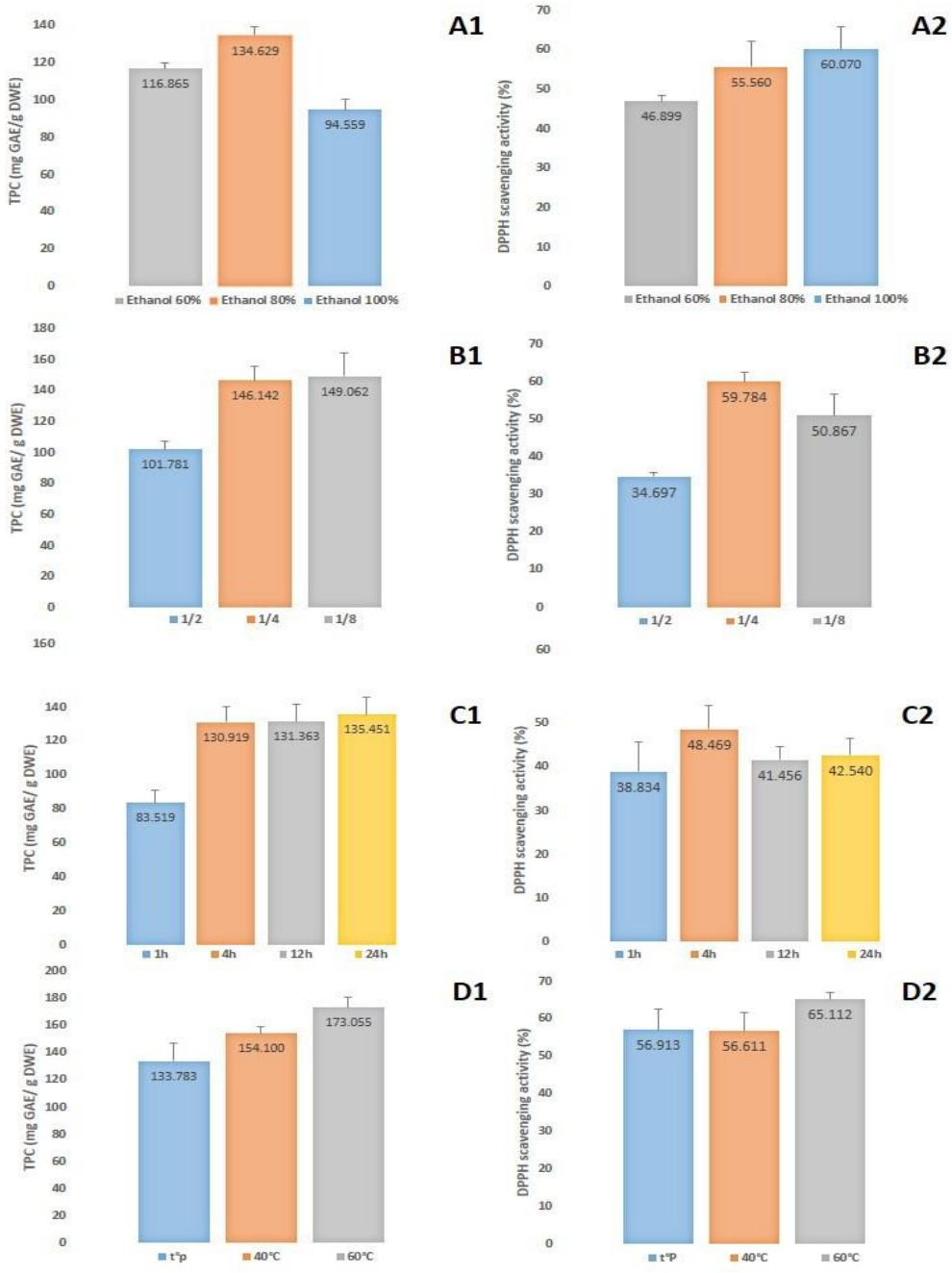

Figure 1 Effects of extraction parameters on TPC and its antioxidant activity of Phu Quoc sim fruits. A1 to D1 present the total phenolic content of extracts. A2 to D2 show the DPPH scavenging percentage of extracts at the concentration of 100µg/ml

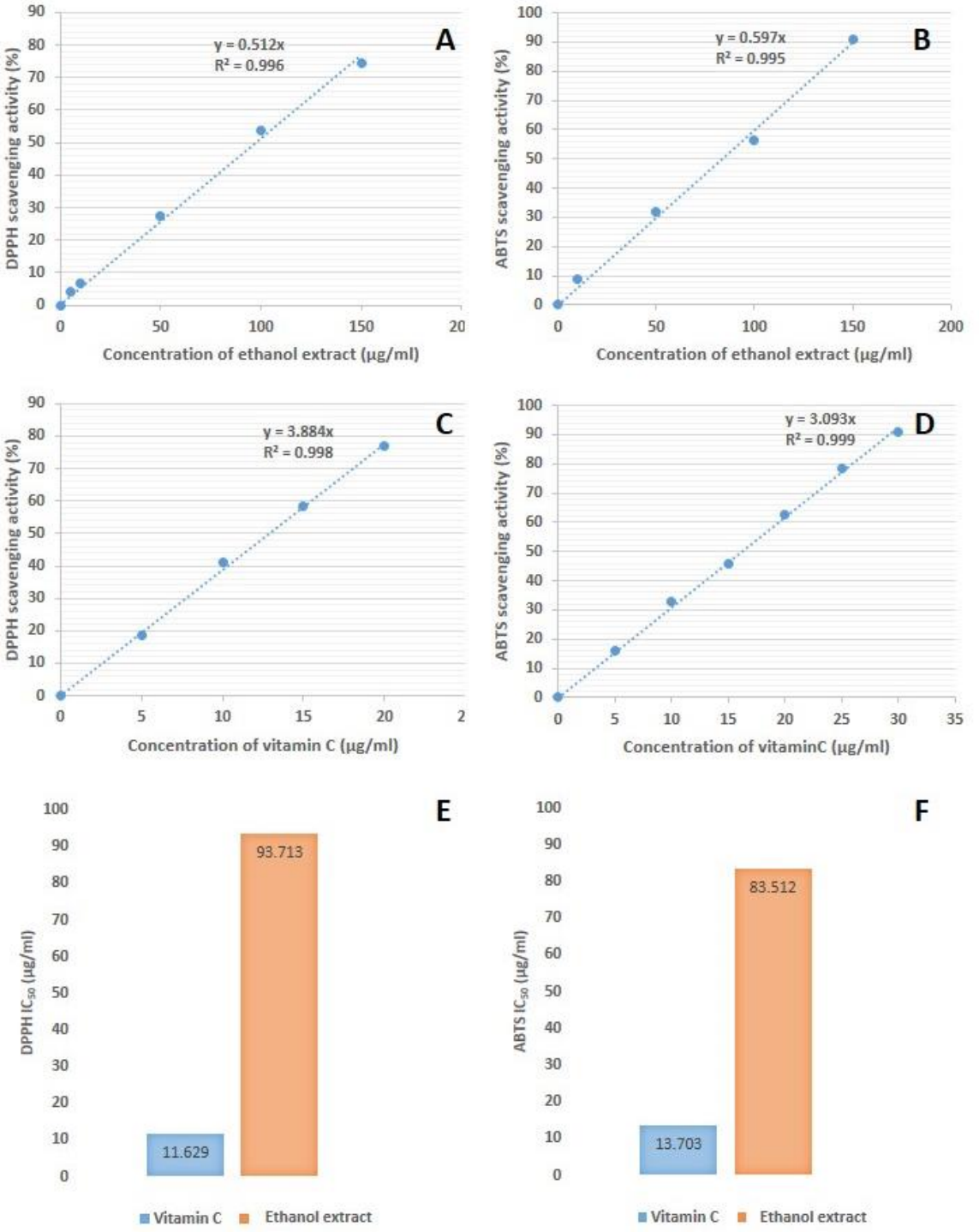

Figure 2 DPPH (A and C) and ABTS (B and D) radicals scavenging activities of ethanol extract and vitamin C. IC_{50} value of ethanol extract and vitamin C on DPPH (E) and ABTS (F) were deduced from the stand curve building by A, B, C and D

3 Results and discussion

3.1 Effect of extraction parameters on TPC and its antioxidant activity

The extraction efficiency is the result of extraction process under many specific conditions. Previous studies have reported the effect of some parameters (temperature, time, SSR, ect.) on total phenolic content[14,16]. They show that the positive or negative effects of each condition during the mass transfer are not always clear. The chemical properties as well as the variety components of natural compounds present different behaviors which are unpredictable.

3.1.1 Effect of aqueous ethanol level

The extraction is critical process for recovering and isolating phenolic compounds from plant materials. Among numerous extraction conditions, the solvent is known as one of the essential parameters for extracting phenolic compounds[22]. In this study, we used ethanol as a solvent because of its safety[10]. Various solutions of ethanol including 60%, 80%, and 100% ethanol have been used for extraction process. As the result, Figure 1A1 showed that there was an increase in TPC content by increasing the ethanol concentration and the highest yield was obtained up to 134.629 mgGAE/g DWE under 80% ethanol. Meanwhile, the increase of solvent concentration from 80% to 100 % reduced the TPC content. Therefore, ethanol 80% was selected for extracting TPC in further investigations. Moreover, the crude extract from ethanol 80% exhibited the efficient antioxidant activity as compared with others via scavenging 55,560% DPPH radicals (Figure 1A2). Therefore, ethanol 80% was applied for the further investigations of optimal extraction conditions.

3.1.2 Effect of SSR

Beside aqueous ethanol level, the effect of SSR value was also subjected for determining the content of TPC. As shown in Figure 1B1, the different SSR of (1:2), (1:4), and (1:8) resulted in different content of TPC with values of 101.784, 146.142, 149.062 mg GAE/g DWE, respectively. It indicates that the TPC content increases following the decrease of SSR. However, the decrease in the SSR value by less than 1/4 did not cause significant changes in TPC content. Moreover, the DPPH scavenging activities of the crude extract (1:4) was 59,784%, which is higher than other ratio groups (Figure 1B2). Thus, the SSR value of (1:4) was subjected for the next investigation.

3.1.3 Effect of extraction time

Herein, the content of TPC and its antioxidant activity was determined depending on various extraction times including 1 h, 4 h, 12 h, and 24 h. The results showed that TPC values are 83.519, 103.919, 131.363, 135.451 mg GAE/g DWE under 1 h, 4 h, 12 h, and 24 h treatments respectively (Figure 1C1). It indicates that TPC has strongly increased in the first 4 hours treatment, suggesting the extraction time significantly effect to the content of TPC. However, the increase in the extraction time from 12 h to 24 h did not cause significant changes in the total polyphenol content. In addition, the longer sample immersion associated with the increase in exposure to heat, oxygen or light which oxidize other antioxidants in the extract , causing a decrease in scavenging ability (Figure 1C2). As the result, the sim fruits powder was recommended to soak in ethanol 80% for 4 h for extraction.

3.1.4 Effect of extraction temperature

The results shown in Figure 1D1 and 1D2 indicate that the TPC content and the antioxidant activity increased with the increase of temperature. Polyphenol has been proved to be very heat-stability compounds [24]. In general, higher temperatures lead to more energy consumption during the extraction process, thus enhancing both the solubility of solution and the diffusion coefficient[7,13]. High temperatures, however, are not always suitable for extracting antioxidant compounds since some antioxidants are unstable and can easily degrade. Moreover, high temperature extraction wastes energy and produces sub-compounds which adversely affect the main products. In this study, three parameters of temperature including room temperature, 40°C, and 60°C were applied for the further evaluation of TPC and its antioxidant activity. Figure 1D1 showed that the content of TPC notably increased when the extraction temperature reached 60°C. The contents of TPC at room temperature and 60°C are 133.783 and 173.055mg GAE/g DWE respectively. In the same line, Figure 1D2 also shows that the DPPH scavenging activity of ethanol extract at 60°C is higher than others. Thus, it recommend that the extraction process should be performed at 60°C.

3.1.5 Recommended processing conditions

From the results obtained by optimizing extract conditions for antioxidant activity and TPC content, we propose the optimal extraction conditions for the effective acquisition of phenolic compounds from Phu Quoc sim as shown in the table 1.

Table 1 The optimization of parameters for efficient extraction

	Ethanol concentration (v/v) (%)	**SSR (w/v)**	**Temperature (°C)**	**Extraction time (hours)**
Recommended parameters	80	1/4	60	4

Under optimal extraction conditions, the content of TPC was achieved with 173.055mg GAE/g DWE. Several studies have been reported on the determination of polyphenol content in medicinal herbs such as green tea (31.6 mg/g DWE) [1] *Dolichandrone spathacea* (52.75 mg GAE/g DWE)[9], carob seeds (25.58 mg GAE/g DWE) [17]. Moreover, Hoang Thi Yen et al. (2015) [24] have reported TPC contents in the ethanol extracts from Hai Duong sim leaf and Hai Duong sim fruit were 104,16 mg GAE/g DWE and 29,23 mg GAE/g DWE which were (1.5-6 times) lower than the TPC content in ethanol extracts from Phu Quoc sim fruits. Accordingly, Phu Quoc sim fruits are considered a great source of bioactive phenolic compounds due to its high TPC.

3.2 DPPH and ABTS scavenging activities of ethanol extract

DPPH and ABTS radicals have absorption band at 490 and 734nm, respectively. They will lose absorption when accepting an electron or a free radical species, which

results in a visually noticeable discoloration. They are sensitive enough to detect active ingredients at low concentrations. Thus, DPPH and ABTS radicals are widely used in assessing free radical scavenging activity of natural products[3]. Moreover, the IC_{50} value of a compound is inversely related to its antioxidant capacity, as it expresses the amount of antioxidant required to scavenging 50% DPPH and ABTS radicals[12]. A lower IC_{50} indicates a higher antioxidant activity of a compound. Figure 2 showed that antioxidant activities of sim fruits extract (Figure 2A and 2B) and Vitamin C (Figure 2C and 2D) increased in a concentration-dependent manner. The IC_{50} values of crude extract in DPPH and ABTS scavenging activities are 93,713µg/ml and 83,512µg/ml, respectively. Meanwhile, the IC_{50} values of vitamin C in DPPH and ABTS scavenging activities are 11,629µg/ml và 13,703µg/ml, respectively (Figure 2E and 2F). The antioxidant activities of ethanol extract from Phu Quoc sim fruits are notably stronger than that of common medicinal plants such as apple pomace (DPPH IC_{50} = 3566µg/ml), *Cassia tora* (DPPH IC_{50} = 102.36µg/ml), *Bauhinia purpurea* (DPPH IC_{50} = 107,94 µg/ml), *Salvia officinalis L.* (ABTS IC_{50} = 210µg/ml)[18,19]. It indicated that sim fruits are potential antioxidant with high application in food industry.

4 Conclusion

In conclusion, this study have determined that the highest TPC content and antioxidant activities of Phu Quoc sim fruits can be achieved at the optimal extraction conditions of ethanol 80% for 4 hours at 60 °C and solid-to-solvent ratio of 1/4 (w/v). The ethanol extract had a TPC content of 173.055 mg GAE/g DWE and DPPH and ABTS scavenging activities with IC_{50} values of 93,713µg/ml và 83,512µg/ml, respectively. The further studies due to purification of single phenolic compounds with high biological activities are necessary for the future application in health-benefit foods.

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Tác động của các điều kiện tách chiết lên hàm lượng phenolic tổng số và hoạt tính kháng oxi hóa của quả sim Phú Quốc (*rhodomyrtus tomentosa* (ait.) hassk.)

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Tóm tắt *Rhodomyrtus tomentosa* là một loại cây bụi thường xanh có nguồn gốc từ Đông Nam Á với hoa màu hồng và trái hình chuông màu tím đậm. Nghiên cứu này tiến hành khảo sát ảnh hưởng của các yếu tố lên quá trình tách chiết nhằm xác định điều kiện tối ưu thu nhận cao chiết có hàm lượng hợp chất phenolic tổng và khả năng kháng oxi hóa tốt nhất nhất từ quả quả sim Phú Quốc. Kết quả cho thấy hàm lượng hợp chất phenolic tổng trong cao quả sim đạt được 179,055mg acid gallic trên 1g trọng lượng cao chiết khô (GAE/DWE) và hoạt động quét gốc tự do DPPH là 65,112% ở nồng độ 100µg/ml. Điều kiện tách chiết tối ưu đã được xác định với ethanol 80%, thời gian 4 giờ, nhiệt độ 40°C và tỉ lệ mẫu/dung môi là 1/4 (w/v). Hoạt tính kháng oxi hóa của cao chiết ethanol thể hiện qua khả năng quét gốc tự do DPPH (IC_{50}=93,713µg/ml) và ABTS (IC_{50}=83,512µg/ml). Theo đó, quả sim Phú Quốc được cho là một nguồn polyphenol tiềm năng có hoạt tính kháng oxi hóa cao.

Từ khóa Sim, *Rhodomyrtus tomentosa*, phenolic, DPPH, kháng oxi hóa