Trop J Nat Prod Res, July 2021; 5(7):1179-1184 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Optimization of Anthocyanin Extraction from *Rhodomyrtus tomentosa* (ait.) Hassk. Fruits and their Antioxidant Potentials

Thanh-Quang Nguyen[1], Huynh D. P. Tran[2], Dang-Khoa Nguyen[1], Hoang N. M. Nguyen[3], Hoang-Khang Lu[1], Dai-Hung Ngo[4]*, Thanh S. Vo[3]*

[1] *Faculty of Technology, Van Lang University, Ho Chi Minh city, Vietnam*
[2]*Department of Biochemistry, Faculty of Biology and Biotechnology, University of Science, Vietnam National University, Ho Chi Minh City, Vietnam*
[3]*NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam*
[4]*Thu Dau Mot University, Binh Duong province, Vietnam*

ARTICLE INFO

Article history:
Received 18 October 2020
Revised 09 June 2021
Accepted 17 July 2021
Published online 02 August 2021

ABSTRACT

Fruits of *Rhodomyrtus tomentosa* (sim fruits) are comprised of high amount of anthocyanin content. They are among the richest sources of anthocyanins, an indication that the plant is endowed with potential bioactive agents. Anthocyanins are plant pigments that play important roles in plant physiology, as well as, beneficial health effects to humans. In this study, optimal conditions for extraction of total anthocyanins of *R. tomentosa* fruits and their antioxidant activity were investigated. Parameters, such as solvent, acid concentration, solid-to-solvent, ultrasound, temperature, and time were applied for investigation of extraction efficiency of anthocyanins of the plant. Total anthocyanin content was determined via the pH differential method. Antioxidant activity of the anthocyanin-rich extract was then examined using DPPH and $ABTS^+$ radical-scavenging assays and DCFH-DA method. The results showed that total anthocyanin content of up to 123.9 mg/kg was obtained from the sim fruits under the extraction conditions of acidified 50% aqueous ethanol by hydrochloric acid solution 0.1 M at a solid-to-solvent ratio of 1:4 with the assistance of ultrasound for 60 min before being processed at a temperature of 60°C for 60 min. The anthocyanins-rich extract was able to scavenge DPPH and $ABTS^+$ radicals with IC_{50} values of 0.0173 ± 0.001 µg/mL and 0.0238 ± 0.0002 µg/mL, respectively. Moreover, the inhibitory activity of the anthocyanin-rich extract on intracellular reactive oxygen species production from macrophage RAW 264.7 cells was also evidenced. As a result, anthocyanins from *R. tomentosa* fruits might be a useful source of ingredients for the prevention of oxidant-induced disorders.

Keywords: Anthocyanins, *Rhodomyrtus tomentosa*, Sim fruits, Antioxidant, Reactive oxygen species.

Introduction

Anthocyanins are polyphenolic pigments responsible for the red, blue, and purple colours to various flowers, fruits, grains, and tubers of terrestrial plants.[1] They exist mainly as anthocyanidin aglycones and anthocyanin glycosides. The anthocyanidins are subdivided into 3-hydroxyanthocyanidins, 3-deoxyanthocyanidins, and *O*-methylated anthocyanidin, while, anthocyanidin glycosides include 3-monoglycosides and 3,5-diglycosides.[2] Anthocyanins are considerably affected by pH of solution; appearing as red in acidic, purple in neutral and blue in alkaline conditions. Their maximum absorption ranges from 520 - 540 nm.[3] Anthocyanin-rich plants have been applied as food colourants instead of synthetic food dyes, functional food ingredients and dietary supplements.[4,5] Traditionally, anthocyanin-rich plant extracts have been used as herbal medicines by Indians, Europeans, and Chinese to treat hypertension, pyrexia, liver disorders, diarrhea, urinary problems, and common cold.[6]

*Corresponding author. E mail: hungnd@tdmu.edu.vn; vtsang@ntt.edu.vn
Tel: +84 76 221 8429; +84 28 62717296

Citation: Nguyen TO, Tran HDP, Nguyen DK, Nguyen HNM, Lu HK, Ngo DH, Vo TS. Optimization of Anthocyanin Extraction from *Rhodomyrtus tomentosa* (ait.) Hassk. Fruits and their Antioxidant Potentials. Trop J Nat Prod Res. 2021; 5(7):1179-1184. doi.org/10.26538/tjnpr/v5i7.2

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The use of anthocyanins as pharmaceutical ingredients has been studied under different *in vitro*, animal, and human models.[1] They act as a strong radical scavenger due to a positive charge at the oxygen atom of the C-ring of basic flavonoid structure.[5] Also, they exhibit a wide range of other biological activities such as anti-cancer, anti-inflammation, anti-diabetes, anti-aging, UV protection, immune system enhancement and eyesight improvements.[1,4,5] Therefore, the consumption of anthocyanins may contribute to the prevention of various diseases, such as; cancer, diabetes, cardiovascular, and neurological disorders. *Rhodomyrtus tomentosa* (Ait.) Hassk belongs to the family *Myrtaceae* and is native to Southern and Southeastern Asia. The fruits of the plant is traditionally used by the Vietnamese, Chinese and Malay in the treatment of diarrhea, dysentery, gynaecopathy, and stomachache.[7] So far, various constituents from *R. tomentosa* fruits have been identified such as triterpenes, steroids, and phenolic compounds.[8,9] Notable, anthocyanins from *R. tomentosa* fruits were also reported in different studies. Liu *et al*.[10] have indicated that maximum yield of anthocyanin from the skin of *R. tomentosa* fruits was achieved up to 4.358 ±0.045 mg/g at the extraction conditions of 60% ethanol containing 0.1% (v/v) hydrochloric acid, 15.7:1 (v/w) liquid to solid ratio, 64.38°C with a 116.88 min extraction time. Although anthocyanin content is mainly present in the skin of *R. tomentosa* fruits, the separation of skin from fruits is impossible in large scale of anthocyanin production. In the study of Cui *et al*.,[11] anthocyanin content in whole fruits of *R. tomentosa* was shown up to 29.4 mg/100 g dry weight via using the extraction solvent of trifluoroacetic acid:methanol (1:99, v/v) for 48 h in the dark at room temperature. However, the extraction conditions under various extraction parameters should be further investigated. The present

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

study was conducted to optimize the extraction conditions for anthocyanin content from *R. tormentosa* fruits and further investigate its antioxidant activity due to scavenging free radicals and inhibiting intracellular reactive oxygen species production in macrophages. The total anthocyanins in foods/plants can be measured by the pH differential method and expressed as cyanidin-3-glucoside equivalent.[10] The principle of this method is due to the change in absorbance of anthocyanidins at respective pH values. It was reported that anthocyanidins can be transformed into flavylium cations at low pH and hemiketal forms at pH 4.5.[12] Since the pH differential method is a rapid and simple method and does not require high-cost instrumentation, it was used for measurement of total anthocyanins in *R. tomentosa* fruits in the present study.

Materials and Methods

Materials

The ripe fruits of *R. tomentosa* were collected from Phu Quoc district, Kien Giang province, Vietnam in July 2019. It was verified by Institute of Tropical Biology (District 3, Ho Chi Minh City, Vietnam) under the number of 14651. Ethanol was purchased from Xilong (China). All other reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Extraction parameters

Whole sim fruits were air-dried under shade and powdered using a grinder. The powder was soaked with solvents under different extract conditions:

(i) Use of 3 different solvents; 50% methanol, 50% ethanol and water and further use of ethanol in varying concentrations of 10%, 30%, 50% and 70%;

(ii) Acidification with 0.1 M each of HCl, acetic and citric acid and further use of different concentrations of HCl (0.05, 0.1, 0.2 and 0.4 M)'

(iii) Ratio of *R. tomentosa* fruit powder-to-ethanol 50% (1:2, 1:3, 1:4, 1:8, 1:12, 1:16);

(iv) Ultrasonic time (15, 30, 60, and 120 min);

(v) Extraction temperature (25, 40, 60, and 80^{o}C) and extraction time (1, 2, 4, and 6 h).

Total anthocyanin content was used as criteria for screening the optimal extract condition. The anthocyanin content was measured via pH differential method. Total anthocyanin content was expressed as milligram anthocyanin per kilogram dried fruit powder (mg/kg).

pH differential method

The pH differential method was performed by spectrophotometric method as previously described by Lee *et al*.[12] The anthocyanin extract solution was diluted in KCl buffer (0.025 M, pH = 1) or CH_3COOH buffer (0.4 M, pH = 4.5) and the absorbance was measured at 520 and 700 nm (Genova Nano, Jenway, UK). The anthocyanin content (mg/kg) was expressed as cyanidin-3-glucoside equation (1):

$$\text{Anthocyanin content} = \frac{\text{A x MW x DF}}{\varepsilon \text{ x l}}\ x\ \frac{V}{m} x 10^3 \quad \ldots\ldots\ldots\ldots (1)$$

Where, A (absorbance) = $(A_{520} - A_{700})_{KCl\ buffer} - (A_{520} - A_{700})_{CH3COOH\ buffer}$; MW (molecular weight of cyanidin-3-glucoside = 449.2 gmol-1); DF = dilution factor; l = pathlength cm; Ɛ = molar extinction coefficient = 26,900 L mol^{-1}cm^{-1}, for cyanidin-3-glucoside; and 10^3 = factor for conversion from g to mg; m = gram of *R. tomentosa* fruits (1 g) in a tested volume (V) of 4 mL.

Radical scavenging assay

Radical scavenging activity of anthocyanin-rich extract solution (AE) was determined by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2´-azinobis-3-ethyl benzothiazoline-6-sulfonic acid (ABTS) assays as previously described.[13] Briefly, AE (1.4 µg/mL) was two-fold serially diluted by distilled water to achieved different concentrations of 0.7 µg/mL, 0.35 µg/mL, 0.175 µg/mL, and 0.0875 µg/mL; and 100 µL of each dilution was mixed with either 100 µL of DPPH or 900 µL of $ABTS^+$ solution. Both mixtures were incubated at room temperature for 30 min (for DPPH) and 6 min (for $ABTS^+$) in the dark and absorbance was then measured at 490 nm (for DPPH) and 734 nm (for $ABTS^+$). Ascorbic acid was used as a reference and diluted by distilled water into different concentrations of 5, 10, 15, 20, 25 µg/mL. The radical scavenging ability was determined using the formula (2), (OD, optical density):

$$\text{Scavenging ability (\%)} = \frac{(\text{ODcontrol} - \text{ODblank}) - (\text{ODAE} - \text{ODblank})}{(\text{ODcontrol} - \text{ODblank})} * 100 \quad \ldots\ldots\ldots\ldots (2)$$

Where, $OD_{control}$ = +DPPH/$ABTS^+$ –AE; OD_{blank} = –DPPH/$ABTS^+$ +AE; OD_{AE} = +DPPH/$ABTS^+$ +AE;

50% inhibitory concentration (IC_{50}) of AE on DPPH and $ABTS^+$ was indicated via radical scavenging ability (%) of AE at a range concentration of 1.4 µg/mL, 0.7 µg/mL, 0.35 µg/mL, 0.175 µg/mL, and 0.0875 µg/mL. Likewise, IC_{50} of Ascorbic acid on DPPH and $ABTS^+$ was indicated via radical scavenging ability (%) of Ascorbic acid at a range concentration of 5, 10, 15, 20, 25 µg/mL.

Reactive oxygen species assay

The production level of reactive oxygen species (ROS) was examined in Ralph and William's cell line 264.7 (RAW 264.7 cells) using a method described by Eruslanov and Kusmartsev.[14] RAW 264.7 cells ($2x10^4$ cells/mL) were incubated with 100 µM dichloro-dihydro-fluorescein diacetate (DCFH-DA) for 45 min in the dark at 37^{o}C. The washed cells were then treated with AE from sim fruits (5 µg/mL) for 60 min before being stimulated with 500 µM H_2O_2 for 5 min. The fluorescence intensity was measured for each period of 15 min at excitation and emission wavelengths of 485 nm and 535 nm, respectively.

Cell viability assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method was applied for cell viability test.[15] Briefly, RAW 264.7 cells (2 x 10^4 cells/mL) were treated with AE (2, 5, and 10 µg/mL) for 24 h. The culture medium was then replaced by 100 µL of MTT solution (0.5 mg/mL) for 4 h at 37^{o}C before adding 100 µL of DMSO. Absorbance was measured at 540 nm and percentage of the cell viability was calculated as compared to the blank group (without AE treatment).

$$\text{\% Cell viability} = \frac{\text{OD(AE)}}{\text{OD(Blank)}}\ x\ 100$$

Statistical analysis

Data were analyzed using the analysis of variance (ANOVA) test of statistical package for the social sciences (SPSS). The statistical differences among groups were assessed by using Tukey's post hoc test. Differences were considered significant at $p < 0.05$.

Results and Discussion

Effect of extraction parameters on anthocyanin content

Effect of solvent type and concentration

Nature and concentration of solvent is among essential parameters for investigation of extraction conditions of total anthocyanin content. Herein, three solvents including water, 50% methanol, and 50% ethanol aqueous solutions were used for anthocyanins extraction from the fruits of *R. tomentosa*. The total anthocyanin content was 8.3, 16.5, and 10.2 mg/kg using 50% methanol, 50% ethanol and water, respectively (Figure 1A). This indicated that 50% ethanol extract was more effective than water and 50% methanol in the anthocyanin extraction. Ethanol as an effective solvent for the extraction of anthocyanins was further investigated using 10%, 30%, 50%, and 70% ethanol. This afforded 0.2, 2.8, 16.4, and 13.4 mg/kg of anthocyanin from sim fruits, respectively (Figure 1B). This further confirms that 50% ethanol extract was effective in extraction of anthocyanins from dried fruit powder. This supports the findings of other studies in which 50% ethanol has been reported for the extraction of anthocyanins from *Vaccinium corymbosum*,[16] black rice[17] and *Prunus salicina*.[18]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Effect of acidification at different concentrations

It has been reported that acidified organic solvent is useful for the extraction of anthocyanins from plants.[19] The addition of an acid can disrupts the cell membranes, thus enhancing anthocyanin extraction and stabilization.[1] In this study, three acids including acetic (0.1M), citric (0.1M), and hydrochloric acid (0.1 M) were used for the assistance of anthocyanin extraction. It was revealed that a total anthocyanin content of 39.3, 16.9 and 12.3 mg/kg was obtained for hydrochloric, acetic and citric acids, respectively (Figure 2A). This indicates that HCl acid was more efficient than acetic and citric acids in the extraction of anthocyanins from the plant. In the same trend, hydrochloric acid was also reported to be effective in the extraction of anthocyanins from *Vaccinium corymbosum*[16] and black rice.[17] Furthermore, various concentrations of HCl (0.075 – 0.4 M) was examined to determine which was best for extraction of anthocyanins from sim fruits. As shown in Figure 2B, the concentration of hydrochloric acid in a range of 0.075 – 0.2 M increased total anthocyanin content from 20.6 mg/kg to 44.8 mg/kg. However, the increase in hydrochloric acid concentration up to 0.4 M reduced total anthocyanin content to 39.5 mg/kg (Figure 2B). In this sense, hydrochloric acid 0.1 M was preferred for the further investigation.

Effect of ratio of R. tomentosa fruit powder-to-ethanol 50%

The effect of ratio of *R. tomentosa* fruit powder-to-ethanol 50% on total anthocyanin content was also investigated in a range of 1:2 – 1:16 (g/ml). The results showed that total anthocyanin content increased in a range ratio of 1:2 (4.7 mg/kg) to 1:8 (44.5 mg/kg). However, there was a decrease in total anthocyanin content at the ratio of 1:16 (39.4 mg/kg) (Figure 3A). Although the highest anthocyanin content was observed at the ratio of 1:8, the ratio of 1:4 (43.6 mg/kg) was preferred to avoid wasting the solvent.

Effect of ultrasonic time

Ultrasound has been widely applied for the extraction of bioactive compounds from plants due to increase in the extraction yield and reduction of extraction time, solvent consumption, and temperature.[19] The ultrasound-assisted extraction has been used to extract anthocyanin from red cabbage (*Brassica oleracea* L. Var. Capitata f. Rubra),[20] Jabuticaba (*Myrciaria cauliflora*) peel,[21] and strawberry (*Fragaria ananassa* Duch).[22] In this study, ultrasound-assisted extraction significantly increased total anthocyanin content up to 53.7 mg/kg within a period of 60 min. However, a prolonged sonication time of up to 120 min exhibited no significant increase in total anthocyanin content (Figure 3B). Thus, ultrasonic period of 60 min was set for the next step of anthocyanin extraction.

Effect of temperature

In general, a higher temperature means more energy consumption during the extraction process and enhances both the solubility and the diffusion coefficient.[23] In order to determine the influence of temperature on anthocyanin extraction, different temperature levels were set at 25 (room temperature), 40, 60, and 80°C. Notably, the increase in temperature up to 60°C remarkably augmented total anthocyanin content (126.7 mg/kg) in the extract solution (Figure 4A). However, total anthocyanin content was reduced to 100.8 mg/kg as the temperature was set at 80°C. This result indicates that the temperature of 60°C could be acceptable for the further extraction process.

Effect of extraction time

Extraction time is an important parameter for optimization process due to economizing energy and cost. In this study, different periods of extraction time ranging from 1-6 h were set for the extraction of total anthocyanin content from *R. tomentosa* fruit powder. It was shown that a maximum extraction time period of 2 h produced the highest yield of total anthocyanin content (123.9 mg/kg), while a prolonged extraction time of up to 4 h and 6 h gradually reduced amount of anthocyanin in the extract solution (Figure 4B). The prolonged extraction time may lead to degradation of anthocyanins due to increase in oxidation.[24] Likewise, it has been reported that longer extraction time significantly decreased total anthocyanin content during 4 h of extraction.[25]

Therefore, the period time of 2 h was preferred for the extraction of anthocyanins from *R. tomentosa* fruit powder.

Recommended optimal conditions

Accordingly, all the investigated parameters affected the extraction efficiency of anthocyanins from *R. tomentosa* fruit powder. The optimal extraction conditions from this study have been revealed to be 50% ethanol acidified with 0.1 M HCl solution at a at a solid-to-solvent ratio of 1/4 with an assistance of ultrasound for 60 min before being processed at a temperature of 60°C in 60 min. A total anthocyanin content of up to 123.9 mg/kg was obtained for the fruits of *R. tomentosa* at such optimal extract conditions. In the same trend, Liu *et al*. have reported that maximum yields of anthocyanins of up to 4.358 mg/g was achieved from dried skin sim fruits using 60% ethanol solution containing 0.1% (v/v) HCl at solid-to-liquid ratio of 1:15.7 (w/v), temperature of 64.38°C, and extraction time of 116.88 min.[10] Due to the different materials used for the extraction of anthocyanins (*R. tomentosa* fruits and *R. tomentosa* fruit skin), the yields of anthocyanin content were incompatible.

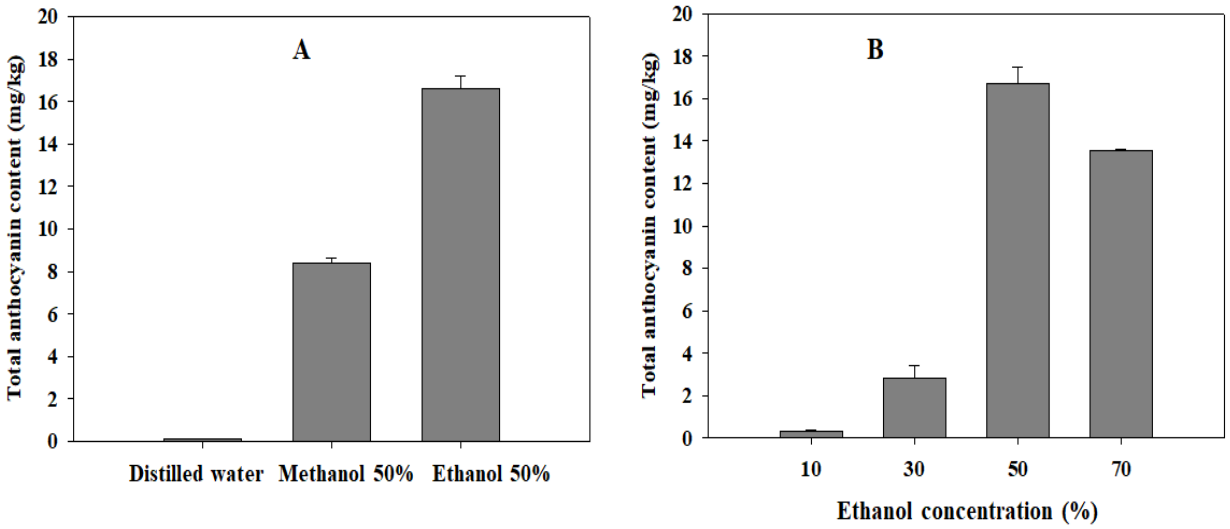

Figure 1: Effect of solvents (A) and different ethanol concentrations (B) on the extraction of total anthocyanin content of *R. tomentosa* fruits. Each determination was made in three independent experiments and the data are shown as means ± SD.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

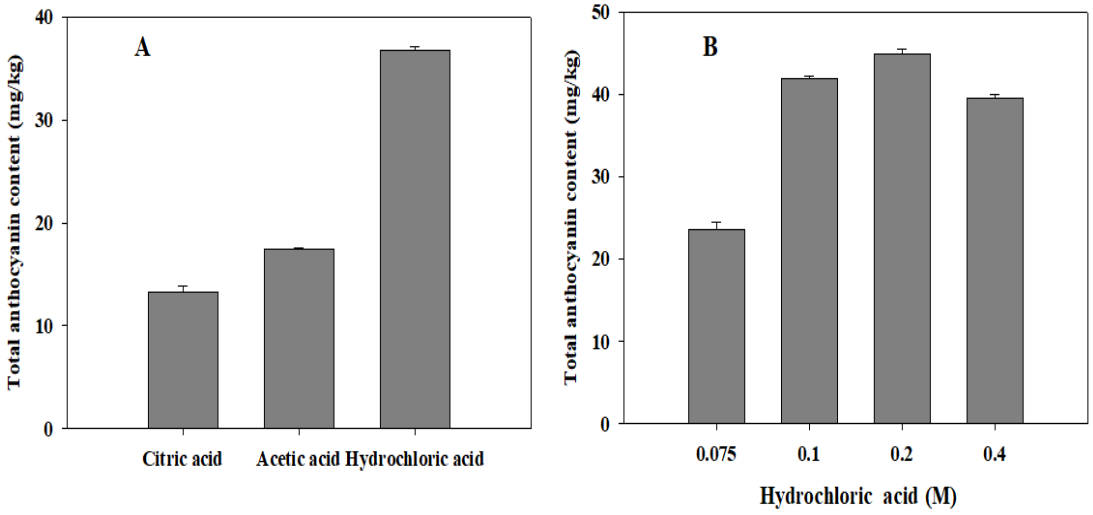

Figure 2: The effect of acids (A) and different concentration of HCl (B) on the extraction of total anthocyanin content from *R. tomentosa* fruits. Each determination was made in three independent experiments and the data are shown as means ± SD.

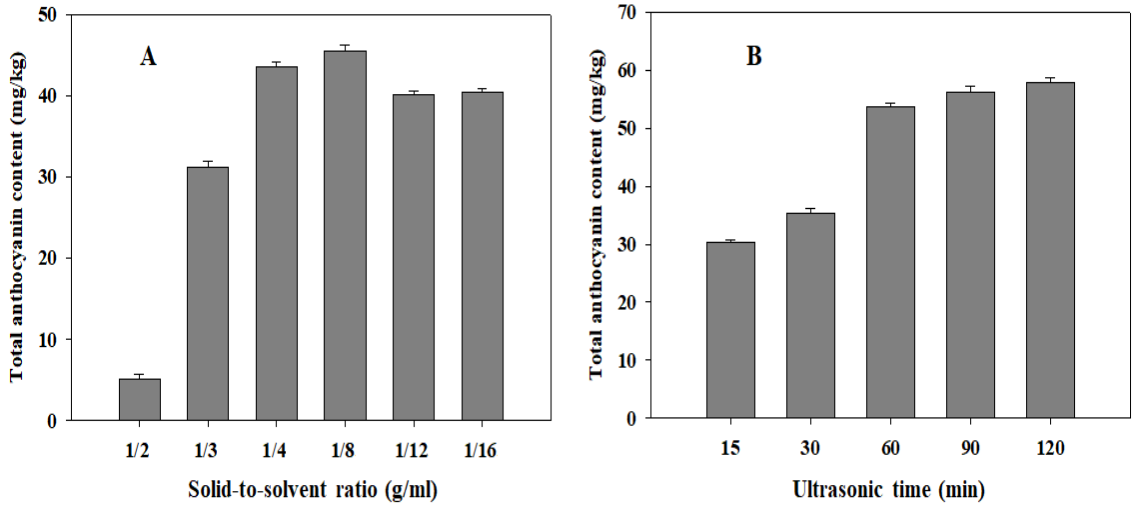

Figure 3: The effect of solid-to-solvent ratio (A) and ultrasonic time (B) on the extraction of total anthocyanin content of *R. tomentosa* fruits. Each determination was made in three independent experiments and the data are shown as means ± SD.

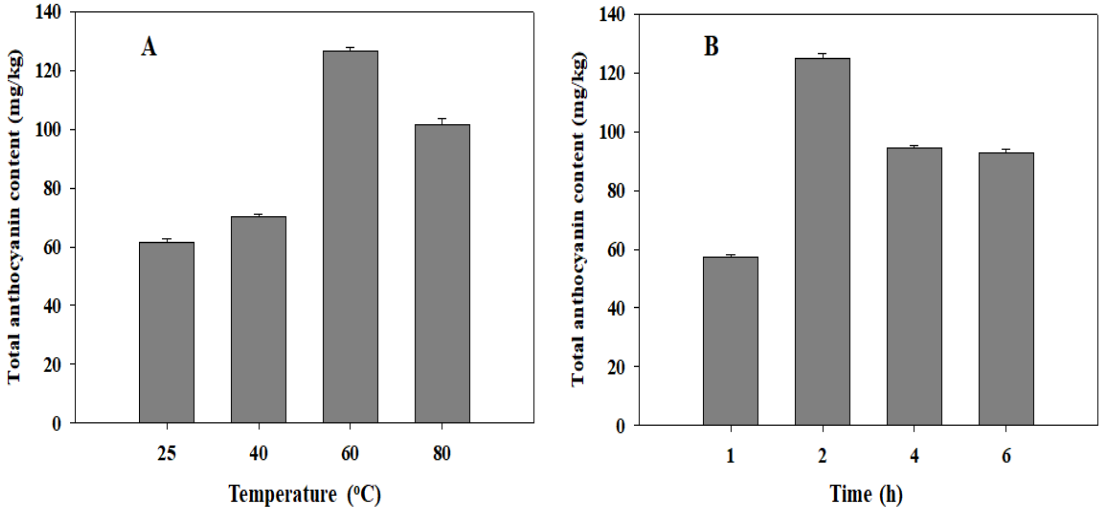

Figure 4: The effect of temperature (A) and time (B) on the extraction of total anthocyanin content of *R. tomentosa* fruits. Each determination was made in three independent experiments and the data are shown as means ± SD.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Free radical scavenging activity

Free radicals are reported to be unstable and reactive chemicals comprising of one or more unpaired electrons in their atoms or molecules.[26] They induce cell damage via passing unpaired electron, causing oxidation of cell components and biomolecules such as lipids, proteins, and DNA.[27] Thus, free radicals have been considered to play a major role in pathogenesis of diseases such as cancer, diabetes, aging, Alzheimer's disease.[28,29] Antioxidants play important functions in neutralization of the free radicals, protection of the cells from radical-induced damages, and prevention of disease pathogenesis.[30,31] Herein, the free radical scavenging activity of anthocyanin-rich extract of *R. tomentosa* fruits (AE) were investigated via DPPH and $ABTS^+$ radical assays. The results showed that AE significantly scavenged DPPH and $ABTS^+$ radicals. The IC_{50} values of AE for DPPH and $ABTS^+$ scavenging activity was to 0.0173 ± 0.001 µg/mL and 0.0238 ± 0.0002 µg/mL, respectively (Table 1). Especially, the free radical scavenging activity of AE was observed to be higher than that of vitamin C, strawberry, blackberry, blueberry, and roselle in Brazil[32,33], raspberries in Poland[34], and Berberis *integerrima bunge* in Iran.[35] These results indicated that AE from *R. tomantosa* fruits is a potential antioxidant agent for prevention of free radicals-induced diseases.

Table 1: Radical scavenging activity of anthocyanin-rich extract of *R. tomentosa* fruits (AE) and vitamin C

Sample	IC_{50} (µg/mL)	
	DPPH	$ABTS^+$
AE	0.0173 ± 0.001	0.0238 ± 0.0002
Vitamin C	21.841 ± 0.041	18.281 ± 0.029

Inhibition of intracellular production of reactive oxygen species

Reactive oxygen species (ROS), including; hydrogen peroxide, superoxide radical, and hydroxyl radical are byproducts of normal metabolic processes in cells.[36] High concentration of these radicals cause cell damage, leading to the pathophysiology of diabetes mellitus, hypertension, cancer, atherosclerosis, and chronic inflammatory diseases.[37] Thus, reduction of ROS production in the cells are suggested for the prevention of such disease. In this study, the inhibitory activity of anthocyanin-rich extract of *R. tomentosa* fruits (AE) on intracellular ROS production from macrophage RAW 264.7 cells was examined. The findings revealed the intracellular ROS level significantly increased from H_2O_2-stimulated cells. Conversely, this increase was mitigated in AE-pretreated RAW 264.7 cells (Figure 5A). Moreover, Figure 5B showed that the AE treatment at a range of 2-10 µg/ml did not cause significant decrease in cell viability, indicating low cytotoxic effect of AE on RAW 264.7 macrophage. Consequently, anthocyanins from sim fruits may contribute to the reduction of intracellular ROS production, ameliorating the oxidative stress in the cells.

Conclusion

In the present study, the optimal conditions for anthocyanin extraction from *R. tomentosa* fruits and its antioxidant activity has been examined. The total anthocyanin content was up to 123.9 mg/kg under optimal extraction conditions. Moreover, anthocyanin-rich extract was shown to possess antioxidant activity via scavenging free radicals and inhibiting intracellular ROS production from macrophages effectively. Hence, anthocyanins from the fruits of *R. tomentosa* were considered as a potential agent for down-regulation of oxidant-induced disorders. However, Further studies should focus on the isolation and characterization of the anthocyanins, as well as, some of their biological activities.

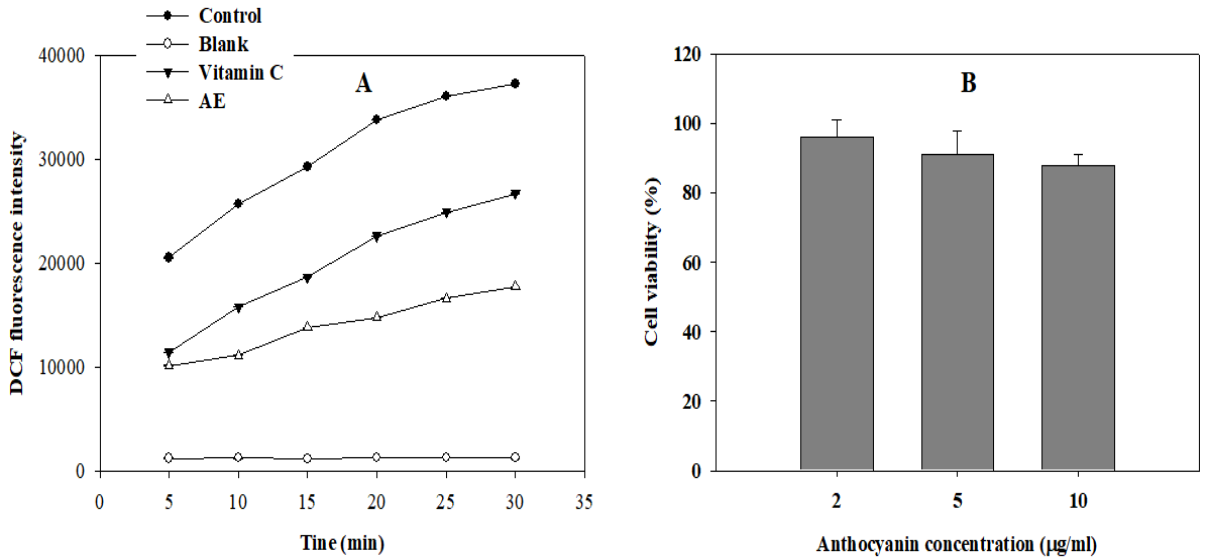

Figure 5: Inhibitory activity of anthocyanin-rich extract (AE) and vitamin C on intracellular production of reactive oxygen species from macrophages (A) and cell viability (B). Each determination was made in three independent experiments and the data are shown as means ± SD; DCF, 2'-7'dichlorofluorescin; Blank = $-H_2O_2$ –AE; Control = $+H_2O_2$ –AE.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This study was supported by The Youth Incubator for Science and Technology Programme, managed by Youth Development Science and Technology Center - Ho Chi Minh Communist Youth Union and Department of Science and Technology of Ho Chi Minh City, the contract number is "42/2019/HĐ-KHCN-VƯ ".

References

1. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res. 2017; 61(1):1-21.
2. Castañeda-Ovando A, Pacheco-Hernández ML, Páez-Hernández ME, Rodríguez JA, Galán-Vidal CA. Chemical studies of anthocyanins: A review. Food Chem. 2009; 113(4):859-871.
3. Laleh GH, Frydoonfar H, Heidary R, Jameei R, Zare S. The effect of light, temperature, pH and species on stability of anthocyanin pigments in four Berberis species. Pak J Nutr. 2006; 5(1):90-92
4. Konczak I and Zhang W. Anthocyanins-more than nature's colours. J Biomed Biotechnol. 2004; 2004(5):239-240.
5. Pojer E, Mattivi F, Johnson D, Stockley CS. The case for anthocyanin consumption to promote human health: a review. Compr Rev Food Sci Food Saf. 2013; 12(5):483-508.
6. Shipp J and Abdel-Aal ESM. Food applications and physiological effects of anthocyanins as functional food ingredients. Open Food Sci J. 2010; 4:7-22.
7. Vo TS and Ngo DH. The health beneficial properties of *Rhodomyrtus tomentosa* as potential functional food. Biomol. 2019; 9(2):1-16.
8. Lai TN, André C, Rogez H, Mignolet E, Nguyen TB, Larondelle Y. Nutritional composition and antioxidant properties of the sim fruit (*Rhodomyrtus tomentosa*). Food Chem. 2015; 168:410-416.
9. Hui WH, Li MN, Luk K. Triterpenoids and steroids from *Rhodomyrtus tomentosa*. Phytochemistry. 1975; 14:833-834.
10. Liu GL, Guo HH, Sun YM. Optimization of the extraction of anthocyanins from the fruit skin of *Rhodomyrtus tomentosa* (Ait.) Hassk and identification of anthocyanins in the extract using high-performance liquid chromatography-electrospray ionization-mass spectrometry (HPLC-ESI-MS). Int J Mol Sci. 2012; 13(5):6292-6302.
11. Cui C, Zhang S, You L, Ren J, Luo W, Chen W, Zhao M. Antioxidant capacity of anthocyanins from *Rhodomyrtus tomentosa* (Ait.) and identification of the major anthocyanins. Food Chem. 2013; 139(1-4):1-8.
12. Lee J, Durst RW, Wrolstad RE. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colourants, and wines by the pH differential method: collaborative study. J AOAC Int. 2005; 88(5):1269-1278.
13. Floegel A, Kim DO, Chung SJ, Koo SI, Chun OK. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. Subtrop Plant Sci. 2011; 24(7):1043-1048.
14. Eruslanov E and Kusmartsev S. Identification of ROS using oxidized DCFDA and flow-cytometry. Methods Mol Biol. 2010; 594:57-72.
15. Mazloum-Ardakani M, Barazesh B, Moshtaghioun SM, Sheikhha MH. Designing and optimization of an electrochemical substitute for the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability assay. Sci Rep. 2019; 9(1):1-8.
16. Oancea S, Stoia M, Coman D. Effects of extraction conditions on bioactive anthocyanin content of *Vaccinium corymbosum* in the perspective of food applications. Procedia Eng. 2012; 42: 489-495.
17. Bae IY, An JS, Oh IK, Lee HG. Optimized preparation of anthocyanin-rich extract from black rice and its effects on *in vitro* digestibility. Food Sci Biotechnol. 2017; 26(5):1415-1422.
18. Joshi VK and Preema DM. Optimization of extraction treatment and concentration of extract on yield and quality of anthocyanins from plum var. 'Santa Rosa'. Indian J Nat Prod Resour 2014; 5(2):171-175.
19. Chemat F, Rombaut N, Sicaire AG, Meullemiestre A, Fabiano-Tixier AS, Abert-Vian M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrason Sonochem. 2017; 34:540-560.
20. Ravanfar R, Tamadon AM, Niakousari M. Optimization of ultrasound assisted extraction of anthocyanins from red cabbage using Taguchi design method. J Food Sci Technol. 2015; 52(12):8140-8147.
21. Kantar SE, Rajha HN, Maroun RG, Louka N. Intensification of polyphenols extraction from orange peels using infrared as a novel and energy saving pretreatment. J Food Sci. 2020; 85(2):414-420.
22. Tiwari BK, O'donnell CP, Patras A, Cullen PJ. Anthocyanin and ascorbic acid degradation in sonicated strawberry juice. J Agric Food Chem. 2008; 56(21):10071-10077.
23. Chaiklahan R, Chirasuwan N, Triratana P, Tia S, Bunnag B. Effect of extraction temperature on the diffusion coefficient of polysaccharides from Spirulina and the optimal separation method. Biotechnol Bioproc E. 2014; 19:369-377.
24. Dai J and Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010; 15(10):7313-7352.
25. Meziant L, Boutiche M, Bey MB, Saci F, Louaileche H. Standardization of monomeric anthocyanins extraction from fig fruit peels (*Ficus carica* L.) using single factor methodology. Food Measure. 2018; 12:2865-2873.
26. Rao PS, Kalva S, Yerramilli A, Mamidi S. Free radicals and tissue damage: role of antioxidants. Free Rad Antiox. 2011; 1(4):2-7.
27. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015; 30(1):11-26.
28. Martínez-Cayuela M. Oxygen free radicals and human diseases. Biochimie. 1995; 77(3):147-161.
29. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008; 4(2):89-96.
30. Pandey KM and Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009; 2(5):270-278.
31. Li S, Chen G, Zhang C, Wu M, Wu S, Liu Q. Research progress of natural antioxidants in foods for the treatment of diseases. Food Sci Hum Wellness. 2014; 3(3-4):110-116.
32. Chaves VC, Boff L, Vizzotto M, Calvete E, Reginatto FH, Simões CM. Berries grown in Brazil: anthocyanin profiles and biological properties. J Sci Food Agric. 2018; 98(11):4331-4338.
33. Wu HY, Yang KM, Chiang PY. Roselle anthocyanins: antioxidant properties and stability to heat and pH. Molecules. 2018; 23(6):1-13.
34. Szymanowska U and Baraniak B. Antioxidant and potentially anti-inflammatory activity of anthocyanin fractions from pomace obtained from enzymatically treated raspberries. Antioxidants. 2019; 8(8):1-13.
35. Farmani F, Moein M, Khoshnood MJ, Sabahi Z. Anthocyanin isolation from berberis *integerrima bunge* fruits and determination of their antioxidant activity. Free Radicals and Antioxidants 2018; 8(1):1-5.
36. Bae YS, Oh H, Rhee SG, Yoo YD. Regulation of reactive oxygen species generation in cell signaling. Mol Cells. 2011; 32(6):491-509.
37. Alfadda AA and Sallam RM. Reactive oxygen species in health and disease. J Biomed Biotechnol. 2012; 2012:1-14.