Antioxidant Activity and Total Phenolic Content of the Crude Extract from *Hibiscus sabdariffa* L.

Charinthorn Fakkham[1,*], Anongnooch thumpad[2], Phanthipha Phuttamek[3], Salinthip Kunsilarak[4] Saengsit Kritsadee[5] Nongnuch Boonjang[6] and Chamiporn Boonsomphan[7].

[1-7]Department of Applied Thai Traditional Medicine, College of Allied Health Sciences, Suan Sunandha Rajabhat University;
anongnooch.tu@ssru.ac.th (A.T.); phanthipha.ph@ssru.ac.th (P.P.); salinthip.ku@ssru.ac.th (S.K.); saengsit.kr@ssru.ac.th (S.K.); nongnuch.bo@ssru.ac.th (N.B.); chamiporn.bo@ssru.ac.th (C.B.)
*Corresponding author: charinthorn.fa@ssru.ac.th (C.F.)

Abstract

Roselle (*Hibiscus sabdariffa* L.; HS) is a widely utilized Thai medicinal plant with a long history of therapeutic use, and it is included in the National List of Essential Herbal Medicines. This inclusion highlights its significance in traditional Thai medical wisdom. The objective of this research was to investigate the antioxidant activity and analyze the total phenolic content of various Roselle parts: the calyx, leaves, and stems. Samples of each plant part, weighing 300 g, were extracted using 95% ethanol and evaporated to dryness using a rotary evaporator to obtain crude extracts for subsequent chemical and biological analysis. Antioxidant activity results showed that the leaf extract exhibited the highest potential, with an IC_{50} value of 0.07 mg/mL. This was followed by the calyx (IC_{50} = 0.18 mg/mL) and the stem (IC_{50} = 0.19 mg/mL).

The standard antioxidant BHT showed an IC_{50} value of 0.01 mg/mL. Regarding the total phenolic content, the leaf and stem extracts contained the highest phenolic levels, both registering 50. 52 ± 0. 003 mg GAE/ mg of dry extract. The calyx followed, with a value of 40.52 ± 0.003 mg GAE/mg of dry extract. This study successfully demonstrates the outstanding antioxidant potential and high phenolic content of Roselle. These findings provide crucial scientific evidence to support the development of cosmeceutical products, skin-rejuvenating formulations, and serve as a foundation for further development of traditional Thai medicinal preparations and future pharmaceutical products.

Keywords: *Hibiscus sabdariffa* L., Antioxidant, Free radical, Total Phenolic Content

1. Introduction

Roselle (*Hibiscus sabdariffa* L.; HS) is a widely recognized medicinal plant belonging to the Malvaceae family, utilized extensively in Thailand for its therapeutic properties. Its importance is affirmed by its inclusion in the National List of Essential Herbal Medicines (Bureau of Herbal Products, 2023), specifically for treating symptoms related to the urinary system (Wright et al., 2007). This incorporation highlights the plant's deep significance in traditional Thai medical wisdom and its established pharmaceutical potential.

Numerous scientific studies have confirmed that Roselle possesses a wide range of pharmacological benefits. Key among these is its ability to reduce blood pressure (Mozaffari-Khosravi et al., 2009; Herrera-Arellano et al., 2004), a function largely attributed to its high content of anthocyanin compounds. Anthocyanins achieve this by inhibiting the synthesis of Angiotensin II, thus decreasing the constriction of blood vessels and leading to reduced blood pressure (Pattanittum et al., 2021). Furthermore, Roselle exhibits various other medicinal properties, including cholesterol lowering, blood sugar regulation in diabetic patients (Wisetmuen et al., 2013), anticancer activity (Ali et al., 2005), and antioxidant effects (Riaz & Chopra, 2018). The antioxidant action of anthocyanins is also known to help reduce the risk of cancer, inhibit tumor growth, and slow down cellular degradation (Tee et al., 1985; Qi et al., 2005; Shipp & Abdel-Aal, 2010). Roselle is highly valued because nearly all parts—the calyx, seeds, and leaves—can be utilized (Siemonsma & Piluek, 1993). The calyx is the most commonly used part, prized for its potent medicinal properties and serving as a rich source of key secondary metabolites, notably anthocyanin, phenolic compounds, and flavonoids (Da-Costa-Rocha et al., 2014; Wisetmuen et al., 2013; Sireeratawong et al., 2013). Antioxidant activity has been reported in crude extracts of the calyx (extracted with both ethanol and water), seeds, and leaves (Wong et al., 2002; Hussein et al., 2010; Mohd-Esa et al., 2010; Anokwuru et al., 2011). Roselle has extensive applications across various industries. In Thailand, it is popularly consumed as a refreshing cold beverage (Thiagarajah et al., 2019) and processed into diverse products, such as ready-to-drink teas (Nanda Yaemphroiprai, 2016; Abo-Baker & Mostada, 2011; Heureux-Calix & Badrie, 2004). It is also employed as a natural food colorant and as an ingredient in cosmetic formulations (Mazza & Miniati, 1993).

Given its reported wide-ranging biological activity, particularly its antioxidant potential—a crucial factor in developing health and anti-aging products (Riaz & Chopra, 2018)—the researchers were thus interested in thoroughly investigating the antioxidant activity and the total phenolic content of various Roselle parts. This study aims to provide scientific data to support the plant's traditional use and accelerate its development and application in the pharmaceutical and cosmeceutical industries.

2. Objective of the Study

1. To evaluate the antioxidant activity of the crude extract obtained from *Hibiscus sabdariffa* L.

2. To determine the total phenolic content of the crude extract obtained from *Hibiscus sabdariffa* L.

3. Methodology

Sample Preparation

The Roselle used in this study was collected from Moo 3, Wang Takhian Subdistrict, Khao Saming District, Trat Province, Thailand, in October 2024. The fresh plant parts (calyx, leaves, and stems) were thoroughly washed with clean water. The cleaned samples were then oven-dried at 60°C until completely dry. The dried plant parts were individually ground into a fine powder.

Extraction process

Powdered samples of the calyx, leaves, and stems were weighed individually at 300 g each. Each 300 g powdered sample was soaked in 600 mL of 95% ethanol, establishing a ratio of 1:2 (w/v). The mixture was extracted using sonication for 30 minutes at room temperature. The sonication step was repeated three times (Chokchaisiri, S., 2025) to ensure complete extraction of the active compounds.
The resulting solution from each individual extraction was filtered through Whatman No. 1 filter paper to separate the plant residue. The filtered extract was then concentrated under vacuum using a Rotary Evaporator to remove the ethanol.
The resulting crude extract was placed in an oven set at 50°C and dried completely to yield a pure crude extract. The dried crude extract was transferred to an airtight container and stored in a freezer at -20°C until further analysis.

Determination of Total Antioxidant Capacity (DPPH Assay)

The total antioxidant capacity of the Roselle extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Thaipong, K., et al. 2006; Noreen, H., et al. 2017; Thamwiriyasati, N., et al. 2022). DPPH is a stable synthetic free radical characterized by its deep purple color. When DPPH reacts with antioxidants, the purple color fades to yellow due to the scavenging activity of the antioxidant, which is dissolved in ethanol.

Preparation of Reagents and Standards

DPPH Solution: 7.9 mg of DPPH powder was accurately weighed and transferred into a 100 mL volumetric flask. The flask was filled with absolute ethanol, shaken until completely dissolved, and the final volume was adjusted to the mark with absolute ethanol Roselle Stock Solution: 10 mg of the Roselle extract was weighed and dissolved in 10 mL of absolute ethanol to yield a stock solution concentration of 1 mg/mL (10,000 µg/mL). BHT Standard Stock Solution 5 mg of Butylhydroxytoluene (BHT) was weighed and dissolved in 5 mL of absolute ethanol to yield a stock solution concentration of 1 mg/mL (1000 µg/mL). Working Solutions (Serial Dilution): The Roselle extract stock and the BHT standard stock were subjected to serial dilution using absolute ethanol to prepare the working solutions at the following concentrations: 1000, 800, 500, 400, 250, 200, 125, 100, 62.5, and 50 µg/mL. The final volume for each diluted concentration was 1 mL.

DPPH Assay Procedure

The assay was performed in a 96-well microplate format : 100 µL of the prepared working solutions (extracts and BHT standard) at various concentrations were loaded into the designated wells. The reaction was initiated by subsequently adding 100 µL of absolute ethanol and 100 µL of the prepared DPPH solution to each well, resulting in a final reaction volume of 300 µL. The mixture was then incubated in the dark for 30 minutes to allow the scavenging reaction to proceed. After incubation, the absorbance was measured at a wavelength of 520 nm using a microplate reader. The measured absorbance values were used to calculate the percentage inhibition (%Inhibition) according to the following equation:

$$\%\ \text{Inhibition}\ (\%I) = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Control OD (DPPH + Solvent)

Sample OD (DPPH + Herbal Extract)

Next, calculate the average % inhibition (%I) for each concentration and perform linear regression to determine the concentration required to 50% inhibitory concentration: IC_{50}

Determination of Total Phenolic Content (TPC)

The Total Phenolic Content (TPC) of the Roselle extract was determined using the Folin–Ciocalteu method (FCR), with absorbance readings taken by a microplate reader.

Analysis of Total Phenolic Content

To determine the total phenolic content, use the Folin-Ciocalteu method and measure the absorbance with a microplate reader at 765 nm. The procedure is as follows: Gallic Acid Standard Solution: Gallic acid (GA) was used as the standard. Working standard solutions were prepared in ethanol at the following concentrations: 100, 80, 50, 40, 25, 20, 12.5, 10, 6.25, and 5 µ/mL. Prepare the herbal extract solution at a concentration of 1 mg/mL in ethanol.

Preparation of 10% Folin-Ciocalteu Reagent (FCR)

Measure 10 mL of Folin- Ciocalteu Reagent (FCR) and transfer it into a 100 mL volumetric flask. Add distilled water and shake well to mix, and adjust the volume to the 100 mL mark for the preparation of 75% sodium carbonate (Na_2CO_3). Weigh 7.5 g of sodium carbonate (Na_2CO_3) and place it into a 100 mL volumetric flask. Add water, shake well to dissolve, and adjust the volume to the 100 mL mark.

Experimental Procedure

Add Sample Extract: Pipette 20 µL of the sample extract into each well of a 96-well plate. Add 100 µL of 10% Folin-Ciocalteu Reagent to each well of the 96-well plate. Shake well, and let it stand in the dark for 5 minutes. Add 80 µL of 75% sodium carbonate (Na_2CO_3) to each well of the 96-well plate. Shake well, and let it stand in the dark for 30 minutes. Measure the absorbance at 765 nm using a microplate reader. Perform the experiment in triplicate and

calculate the average. Compute the total phenolic content in the extract, expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g). Plot the absorbance values obtained against a standard calibration curve of gallic acid and perform linear regression. From the gallic acid standard curve, determine the concentration of phenolic compounds in the sample. Express the total phenolic content as milligrams of gallic acid equivalent (GAE) per gram of extract (mg GAE/g).

Calculation Formula

TPC (% w/w GAE) =PS x V x D x 10^{-6} x 100/W

PS = phenolic content of the solution (µg/ml)

V = Total volume of sample (mL)

D = dilution factor

W = sample weight (g)

4. Results

Data Analysis

In this study, each sample was tested in triplicate (n=3). The average value ($\bar{x}$) and standard deviation (S.D.) were calculated. Differences between groups were compared using ANOVA with a 95% confidence level.

The percentage yield of the crude extract relative to the weight of the plant material (% yield) of Roselle (*Hibiscus sabdariffa* L.) parts using 95% ethanol as the solvent is presented in Table 1.

Table 1: Percentage Yield of Crude Extract

Common name	scientific name	part of used	Sample Weight (g)	Crude Extract Weight (g)	% yield (w/w) 95% Ethanol
Roselle	*Hibiscus sabdariffa* L.	Calyx	300	76.63	25.54
		Leaf	300	59.30	19.77
		Stem	300	6.75	2.25

The extraction results using 95% ethanol indicated a clear difference in yield among the plant parts. The Calyx yielded the highest amount of crude extract, 76.63 g, corresponding to the highest percentage yield of 25.54 g. This was followed by the Leaf, which yielded 19.77 g. Conversely, the Stem showed a significantly lower extraction efficiency, yielding only 2.25 g. of the crude extract.

Antioxidant Activity Testing Using the DPPH Radical Scavenging Assay

The antioxidant activity of Roselle (*Hibiscus sabdariffa* L.) extract and the standard antioxidant, Butylhydroxytoluene (BHT), was determined by the DPPH radical scavenging assay and expressed as the 50% inhibitory concentration (IC_{50}). The results are presented in Table 2.

Table 2 shows the 50% inhibition values for oxidation (50% inhibitory concentration: IC_{50})

Sample	IC_{50} values of DPPH radical scavenging activity (mg/ml)
Hibiscus sabdariffa L. Extract	
Calyx	0.18
Leaf	0.07
Stem	0.19
BHT (Standard)	0.01

The synthetic standard BHT exhibited the highest scavenging activity with the lowest IC_{50} value of 0.01 mg/mL. Among the Roselle extracts, the Leaf extract demonstrated the strongest antioxidant activity (IC_{50} of 0.07 mg/mL.) The Calyx extract (IC_{50} = 0.18 mg/mL) and the Stem extract (IC_{50} = 0.19 mg/mL) showed the weakest activities, indicating that the antioxidant compounds were most concentrated in the leaf portion.

Determination of Total Phenolic Content

Results of Total Phenolic Content Analysis, The total phenolic content of Roselle (*Hibiscus sabdariffa* L.) extract was determined and compared with the standard gallic acid (R^2 = 0.9998), The analysis confirmed the presence of total phenolic content in the extract, and the results are summarized in Table 3.

Table 3: Total Phenolic Content of Hibiscus sabdariffa L. Extract

Extract	Total Phenolic Content (mg GAE/mg of dry extract)
Calyx	40.52 ± 0.003
Leaf	50.52 ± 0.003
Stem	50.52 ± 0.003

The total phenolic content is expressed in milligrams of gallic acid equivalents per milligram of dry extract (mg GAE/mg), indicating the concentration of phenolic compounds in the sample. The high R^2 value (0.9998) reflects the accuracy and reliability of the calibration curve used for quantification.

The analysis revealed that the Leaf and Stem extracts contained the highest amount of total phenolic compounds, both yielding a TPC of 50.52 ± 0.003 mg GAE/mg. The Calyx extract showed a slightly lower TPC value of 40.52 ± 0.003 mg GAE/mg. These findings suggest a high concentration of phenolic compounds across all parts of the *H. sabdariffa* L. extracts.

5. Conclusion

The results This study successfully determined the extraction yield, antioxidant capacity , and total phenolic content of the calyx, leaf, and stem of Roselle (*Hibiscus sabdariffa* L.) using 95% ethanol extraction. Collectively, the leaf extract emerged as the most promising source for natural antioxidants due to its superior radical scavenging activity (lowest IC_{50}), which strongly correlates with its high TPC. These findings suggest that Roselle leaves, in addition to the commonly utilized calyx, should be considered a valuable source for functional food ingredients or natural health products.

Acknowledgements

The author sincerely thanks Suan Sunandha Rajabhat University, Bangkok, Thailand, for their support and extends gratitude to the Science and Technology Services Center and the College of Allied Health Sciences for providing essential facilities throughout this work.

References

Abo-Baker, A. A., & Mostada, A. M. (2011). Effect of some processing treatments on the physicochemical and sensory quality characteristics of roselle (*Hibiscus sabdariffa* L.) beverages. *Alexandria Journal of Agricultural Research, 56*(1), 143–157.

Ali, B. H., Al Wabel, N., & Blunden, G. (2005). Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: A review. *Phytotherapy Research, 19*(5), 369–375.

Anokwuru, C. P., Tarawou, P. O., Adeleye, S. A., & Ogunbayo, B. S. (2011). Isolation, purification and oxidative activity of Roselle Anthocyanins (*Hibiscus sabdariffa* Linn). *Research Journal of Phytochemistry, 5*(2), 85–92.

Chokchaisiri, S., Sripan, P., Yongram, C., Wongsonthom, S., Chimpalee, P., Thomprasert, P., & Chaiphongpachara, T. (2025). Chemical composition and antioxidant activity of the Kae Lom Kae Sen Thai herbal medicinal formula. *Tropical Journal of Natural Product Research, 9*(6), 2632–2644.

Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I., & Heinrich, M. (2014). *Hibiscus sabdariffa* L. – A phytochemical and pharmacological review. *Food Chemistry, 165*, 424–443.

National List of Essential Herbal Medicines B. E. 2566. Retrieved from https://herbal.fda.moph.go.th/media.php?id=553111484570411008&name=nloehd3-03.pdf

Heureux-Calix, D., & Badrie, N. (2004). Consumer acceptability of roselle (*Hibiscus sabdariffa* L.) beverages. *Food Service Technology, 4*(2), 65–75.

Herrera-Arellano, A., Flores-Romero, S., Chávez-Soto, M. A., & Tortoriello, J. (2004). Effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* in patients with mild to moderate hypertension: A controlled and randomized clinical trial. *Phytomedicine, 11*(5), 375–382.

Hussein, R. M., Shahein, Y. E., El Hakim, A. E., & Awad, H. M. (2010). Biochemical and molecular characterization of three colored types of roselle (*Hibiscus sabdariffa* L.). *Journal of American Science, 6*(12), 726–733.

Kakatum, N. (2024). Antioxidant activity and phenolic compounds and determination of quercetin as a biomarker using high-performance liquid chromatography of *Butea monosperma* (Linn.). *ICBTS 2024 Conference in Paris*, 61–69.

Mazza, G., & Miniati, E. (1993). *Anthocyanins in Fruits, Vegetables, and Grains*. CRC Press.

Mohd-Esa, N., Hern, F. S., Ismail, A., & Yee, C. L. (2010). Antioxidant activity in different parts of roselle (*Hibiscus sabdariffa* L.) extracts and potential exploitation of the seeds. *Food Chemistry, 122*(4), 1055–1060.

Mozaffari-Khosravi, H., Jalali-Khanabadi, B. A., Afkhami-Ardekani, M., Fatehi, F., & Noori-Shadkam, M. (2009). The effects of sour tea (*Hibiscus sabdariffa*) on hypertension in patients with type II diabetes. *Journal of Human Hypertension, 23*(1), 48–54.

Pattanittum, P., Ngamjarus, C., Buttramee, F., & Somboonporn, C. (2021). Roselle for hypertension in adults. *Cochrane Database of Systematic Reviews, 11*(11), CD007894.

Qi, Y., Chin, K. L., Malekian, F., Berhane, M., & Gager, J. (2005). Biological characteristics, nutritional and medicinal value of roselle, *Hibiscus sabdariffa*. *Circular - Urban Forestry Natural Resources and Environment, 604*, 1–2.

Riaz, G., & Chopra, R. (2018). A review on phytochemistry and therapeutic uses of *Hibiscus sabdariffa* L. *Biomedicine & Pharmacotherapy, 102*, 575–586.

Shipp, J., & Abdel-Aal, E. S. M. (2010). Food applications and physiological effects of anthocyanins as functional food ingredients. *Open Food Science Journal, 4*, 7–22.

Siemonsma, J. S., & Piluek, K. (Eds.). (1993). *Plant Resources of South-East Asia No. 8*. Pudoc Scientific Publishers.

Sireeratawong, S., Itharat, A., Khonsung, P., Lertprasertsuke, N., & Jaijoy, K. (2013). Toxicity studies of the water extract from the calyces of *Hibiscus sabdariffa* L. in rats. *African Journal of Traditional, Complementary and Alternative Medicines, 10*(2), 122–127.

Tee, S. P., Lim, S. P., & Ooi, C. L. (1985). *Extraction and stabilization of roselle pigment* (Unpublished thesis/dissertation). Universiti Pertanian Malaysia.

Thiagarajah, K., Ong, M. K., Teh, L. K., & Lye, H. S. (2019). Plants infused water as preferred healthy drinks. In *Bottled and Packaged Water: The Science of Beverages* (Vol. 4, pp. 367–402). Woodhead Publishing.

Wisetmuen, E., Pannangpetch, P., Kongyingyoes, B., Kukongviriyapan, U., Yutanawiboonchai, W., & Itharat, A. (2013). Insulin secretion enhancing activity of roselle calyx extract in normal and streptozotocin-induced diabetic rats. *Pharmaceutical Research, 5*(1), 65–70.

Wong, P. K., Yusof, S., Ghazali, H. M., & Man, Y. B. C. (2002). Physico-chemical characterization of roselle (*Hibiscus sabdariffa* L.). *Nutrition and Food Science*, *32*(2), 68–73.

Wright, C. I., Van-Buren, L., Kroner, C. I., & Koning, M. M. (2007). Herbal medicines as diuretics: A review of the scientific evidence. *Journal of Ethnopharmacology*, *114*(1), 1–31.