

Effects of royal lotus petals on antioxidants of bael-rosella mixed herbal drink

*Kamdaeng, O. and Singkaew, K.

*Department of Food and Nutrition, Faculty of Home Economics Technology,
Rajamangala University of Technology Thanyaburi, Pathum Thani, 12110, Thailand*

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Abstract

This research aimed to study the quantity of royal lotus petals and the antioxidant activity in the bael-rosella mixed herbal drink. The recipe of the bael-rosella mixed herbal drink included dried bael, dried rosella, sugar and water at 8.55, 0.85, 5.13 and 85.47%, respectively. The drink was comprised of various amounts of royal lotus petals at 1.25, 1.5, 2.0 and 2.5%. The results showed that 2.0% of royal lotus petals in the mixed herbal drink contained 10°Brix. Regarding the acceptance of the consumers, 2.0% of the lotus petals in the mixed herbal drink recipe was highly satisfied. The antioxidant activity of dried royal lotus petals in the bael-rosella mixed herbal drink showed the amounts of DPPH, metal chelation (MC₅₀) and inhibition of lipid peroxidation (LC₅₀) at 0.91±0.01 mg/mL, 10.79±1.76 mg/mL and 11.96±1.21 mg/mL, respectively. Hence, the bael-rosella mixed herbal drink added with royal lotus petals can be considered as a healthy herbal drink.

1. Introduction

Herbal drinks are the juice that made from several parts of plants, such as flowers, leaves, roots, fruits, seeds, grasses and grains. They are known as thirst-quenching drinks. Herbal drinks can be drunk hot and cold. Additionally, they are rich in vitamins, minerals, antioxidants, and they contain many healthy substances. Since the trend of healthy eating and exercising is popular in Thailand nowadays, herbal drinks are what people have a hunger for. Therefore, a demand for herbal drinks is increasing gradually with a market value of up to 2,700 million baht and a growth rate of 10-15% per year (Ministry of Industry Thailand, 2004).

In Thailand, there are many kinds of flowers that provide beautiful colours and fragrances, and some of them are edible. According to the study of Shi *et al.* (2008), edible flowers contain antioxidants and phenolic substances that can slow down the aging process. It is also perceived as the anti-cancer agent and it prevents Alzheimer's and coronary artery disease. Thus, the consumption of edible flowers can be considered as an alternative option of a healthy lifestyle because these flowers contain the substances that can be identified as "antioxidants".

Huabbangyang (2010) found that red pigments in flowers contain food nutrients and they have a higher

antioxidant capacity than that of white flowers. In fact, red flowers have more substances, namely beta-carotene, flavonoid and anthocyanin than white flowers. As a consequence, blooming red lotus, especially harvested in the morning, contains high nutrients and high antioxidant ability.

Lotuses usually bloom around August to December. This kind of flower has many medicinal properties, such as improving heart health, maintaining strength, nourishing strength, nourishing pregnancy, reducing fever, reducing blood sugar, and containing high fibers. Lotus flowers contain alkaloids called *nelumbine*. Lotus embryos contain lotusine. Lotus pollens contain many flavonoids, such as quercetin, isoquercitrin, luteolin, luteolin glucoside, and alkaloids (Siriruk, 2009). However, royal lotus petals have an astringent taste that comes from tannins. Thus, people usually eat them with other vegetables to reduce the astringent taste. Also, royal lotus petals can be boiled until they give a mild fragrance to make herbal juice. For these reasons, royal lotus petals are added in the bael-rosella mixed herbal drink, which is a popular herbal drink based on the data derived from the consumer survey. Importantly, the colours from royal lotus petals do not change the colour of the drink (Kamdaeng, 2015).

Bael fruit is known as ancient herbal medicine.

*Corresponding author.

Email: ONUMA_K@rmutt.ac.th

Unripe bael fruit can be brewed and mixed with water. People usually drink it as a remedy for diarrhea, dysentery, and gastritis, and it helps people to have a good appetite. Bael fruit can also be used as a stomachic mixture, carminative, and analeptic. Moreover, it can cure chronic intestinal diseases in children. Ripe bael fruit has sweet and astringent tastes. It can be used as dysentery and phlegm remedy. It is also a carminative and a laxative that can maintain gastric ulcers, so it is suitable for the elderly who often suffer from constipation. Furthermore, it can cure inner heat and thirst, sharp pain in a stomach, chronic dysentery, and reduce blood sugar level (Homhual, 2017).

Rosella's sepals are thick, red, and juicy, and people usually regard this part as a flower. The medicinal properties of rosellas are varied. For example, it can relieve dryness and thirst, strengthen immunity, and prevent common colds. In fact, rosellas contain anthocyanin or the red substance that can be found in the blueberries group. Rosellas can reduce fever and treat gastritis and enteritis. The medicine can be made by grinding dried rosellas into powder. It can be taken 1 tablespoon of the powder and followed by a glass of water. It is suggested that people should drink it 3-4 times a day. It is a remedy that can help to heal stomach ulcers and prevent the occurrence of peptic ulcers.

Based on the information mentioned above, the objective of this research was to compare the effects of fresh and dried royal lotus petals in the bael-rosella mixed herbal drink in relation to the antioxidant activity.

2. Materials and methods

2.1 Raw materials preparation

2.1.1 Fresh royal lotus petals

Fresh royal lotus petals were washed thoroughly and dried. Then, the petals were cut into 1 × 3 cm pieces.

2.1.2 Dried royal lotus petals

Dried royal lotus petals were washed thoroughly and dried. After that, they were cut into 1 × 3 cm pieces, and then they were dried at 60°C for 4 hrs.

2.1.3 Bael fruits

Roast bael fruit was heated until it was fragrant.

2.2 Bael-rosella mixed herbal drink preparation

Approximately, 0.8% of dried rosella, 8.55% of bael fruit and 5.13% of sugar were boiled for 30 mins and then filtered.

A total of four concentrations of fresh royal lotus petals at 1.5, 2.0, 2.5 and 3.0% were used in Bael-rosella mixed herbal drink. Then, another three amounts of dried royal lotus petals at 0.4, 0.8 and 1.25% were also used in the drink. The data collection was designed by applying the Completely Randomized Design (CRD) to measure the colour of the drink, and the sensory quality testing with the 9-point Hedonic Scaling (1 = dislike the most, and 9 = like the most), with the Randomized Complete Block Design (RCBD). The samples were analyzed in triplicate, and the sensory test was conducted with the consumers. The variance was analyzed by using ANOVA at the 95% confidence level. The differences were analyzed by using the Duncan's Multiple Range Test.

2.3 Antioxidant activity

An analysis of the antioxidant activity of the bael-rosella mixed herbal drink added with royal lotus petals was conducted with the free radical scavenging activity using the DPPH method, metal chelation activity and lipid peroxidation activity (Kim *et al.*, 2008; Boonpisuttinant *et al.*, 2014).

2.3.1 Free radical scavenging activity using the DPPH method

The samples and vitamin C at concentrations of 0.01, 0.1, 1, 10 and 100%/mL. were prepared, and each sample solution of 50 µl concentration was mixed with vitamin C in a 96-well plate. The samples were shaken well to mix the substances and kept in the dark for 30 mins. After that, the microplate reader absorption measurement at 560 nm wavelength was used to calculate the antioxidant activity (Boonpisuttinant *et al.*, 2014).

2.3.2 Lipid peroxidation activity

The samples were prepared with vitamin E at concentrations of 0.01, 0.1, 1, 10 and 100%/mL. Each sample solution of 50 µl concentrations was mixed with vitamin E at the in a 96-well plate. A solution of 1 mg/mL of linoleic acid emulsion was dissolved in 50% DMSO of 50 µl. Then, 1 mg/mL solution of NH₄SCN was dissolved in 1% HCl of 50 µl and shake well. It was then placed in the dark for 60 mins. The microplate reader absorption measurement set at 450 nm wavelength was used to calculate the inhibition of fat percentage reaction (Boonpisuttinant *et al.*, 2014).

2.3.3 Metal chelation activity

The samples were prepared with EDTA at concentrations of 0.01, 0.1, 1, 10 and 100%/mL. Each sample solution of 50 µL was mixed with EDTA in a 96-well plate. Then, ferrozine of 50 µL was filled into the

samples. Concentrated FeCl₂ solution at 1 mg/mL was then dissolved in 1% HCl, and then of the mixed solution at the amount of 50 µL was shake well. Then, it was kept in the dark for 60 mins. The microplate reader imports light absorption at 560 nm wavelength was used to calculate the metal chelation (Boonpisuttinant *et al.*, 2014).

3. Results and discussion

3.1 Bael-rosella mixed herbal drink preparation

According to the study, the bael juice mixed with 0.8% dried rosella was added with 1.5, 2.0, 2.5 and 3.0% of fresh royal lotus petals. Also, the mixed herbal drink was added with 0.4, 0.8 and 1.25% of dried royal lotus petals. The results showed that the appropriate amounts of fresh royal lotus petals and dried royal lotus petals were 2.0% and 0.8%, respectively. The results are presented in Table 1.

Table 1. Recipe of the bael-rosella mixed herbal drink protocol

Ingredients	Amounts	
	Grams	Percentage
Dried bael fruit	100	8.55
Dried rosella	10	0.85
Sugar	60	5.13
Water	1000	85.47
Total	1170	100

Using 2.0% of fresh lotus petals, the colour measured was $L = 22.9$, $a = 6.7$, $b = 3.3$ and the sweetness was 10% Brix, which was the right amount to produce a quince herb drink with roselle with fresh lotus petals. The consumers gave the highest overall preference score to this drink amongst the four samples. This bael-rosella mixed herbal drink had a better appearance and beautiful colour. The fragrance, taste and sweetness were modest, and the consumers accepted it as a suitable recipe of fresh royal lotus petals for the bael-rosella mixed herbal drink. The results are presented in Table 2.

Table 2. Statistical analysis of mean scores from the sensory observation of the amounts of fresh royal lotus petals in four recipes of the bael-rosella mixed herbal drink

Sensory characteristics	Amounts of fresh royal lotus petals (%)			
	1.5	2	2.5	3
Appearance ^{ns}	6.85±0.18	6.98±0.18	7.13±0.18	6.70±0.18
Brightness	6.58±0.18 ^b	7.22±0.18 ^a	6.95±0.18 ^a	6.73±0.18 ^a
Colour ^{ns}	7.20±0.17	6.78±0.17	7.13±0.17	6.98±0.17
Smell ^{ns}	6.62±0.21	6.72±0.21	6.68±0.21	6.78±0.21
Sweetness	7.15±0.19 ^a	6.45±0.19 ^b	7.02±0.19 ^a	6.33±0.19 ^b
Overall liking	7.13±0.17 ^{ab}	6.75±0.17 ^{ab}	7.28±0.17 ^a	6.58±0.17 ^c

Values are expressed as mean±SD. Values with different superscript within the same row are significantly different ($p \leq 0.05$).

^{ns}Values within the same row are not significantly different ($p > 0.05$).

Using 0.8% of dried lotus petals, the colour measured was $L = 22.9$, $a = 6.7$, $b = 3.3$, and the sweetness was 10% Brix, which was the right amount to produce a quince herbal drink with roselle with dried lotus petals. The consumers gave the most overall preferences amongst the three samples. This bael-rosella mixed herbal drink with dried lotus petals had a better appearance, brightness and beautiful colour. The fragrance, taste and sweetness of the drink appeared to be modest, and it was accepted by the consumers that it was a suitable recipe for the dried royal lotus petals for the bael-rosella mixed herbal drink. The results are presented in Table 3.

Table 3. Statistical analysis of mean scores from the sensory observation of the amounts of dried lotus petals in three recipes of the bael-rosella mixed herbal drink

Sensory characteristics	Amounts of dried royal lotus petals (%)		
	0.4	0.8	1.25
Appearance	6.05±0.15 ^b	7.75±0.15 ^a	7.77±0.15 ^a
Brightness	6.37±0.17 ^b	7.45±0.17 ^a	7.88±0.17 ^a
Colour	6.27±0.17 ^b	7.45±0.17 ^a	7.45±0.17 ^a
Smell	6.77±0.18 ^b	7.78±0.18 ^a	6.85±0.18 ^b
Sweetness	6.25±0.21 ^b	7.08±0.21 ^a	6.97±0.21 ^a
Overall liking	6.50±0.16 ^b	7.32±0.16 ^a	7.40±0.16 ^a

Values are expressed as mean±SD. Values with different superscript within the same row are significantly different ($p \leq 0.05$).

3.2 Antioxidant activity

Antioxidant activity in the bael-rosella mixed herbal drink added with royal lotus petals was tested by applying the methods of DPPH, Metal chelation activity, and Lipid peroxidation activity. In the first method, the experiment using the free radical scavenging activity showed that the bael-rosella mixed herbal drink added with royal lotus petals with the 100% v/v intensity contained the antioxidant activity three times higher than that of 0.1 mg/mL of vitamin C solution. The radical scavenging activity was at 0.37±0.13 mg/mL with a statically significant difference ($p < 0.05$), as shown in

Table 4. Results of the antioxidant ability in the bael-rosella mixed herbal drink added with royal lotus petals

Extraction	Antioxidant (mg/mL)	Antioxidant	
		Metal chelating activity (MC ₅₀) (mg/mL)	Inhibition of Lipid peroxidation activity (LC ₅₀) (mg/mL)
The bael-rosella mixed herbal drink	0.53±0.01 ^c	6.96±0.58 ^d	2.30±0.03 ^b
The bael-rosella mixed herbal drink with fresh lotus petal	0.79±0.01 ^a	9.44±1.99 ^a	2.73±0.13 ^b
The bael-rosella mixed herbal drink with dried lotus petal	0.91±0.01 ^b	10.79±1.76 ^a	11.96±1.21 ^a
Vitamin C	0.37±0.13 ^d	-	-
EDTA	-	0.93±0.37 ^c	-
Vitamin E	-	-	0.35±0.08 ^c

Values are expressed as mean±SD. Values with different superscript within the same row are significantly different ($p \leq 0.05$).

Table 4.

For the next method of Metal chelation activity, the ferrous metal chelating method was used to find the value of MC₅₀. The relationship of metal chelating activity and the concentration (mg/mL) of the bael-rosella mixed herbal drink added with fresh royal lotus petals caused the ability to chelate metal MC₅₀ at 9.44±1.99 mg/mL, while the ability to chelate metal MC₅₀ of the bael-rosella mixed herbal drink added with dried royal lotus petals was at 10.79±1.76 mg/mL. The data also revealed that the bael-rosella mixed herbal drink added with fresh brown royal lotus petals had an ability to chelate metal MC₅₀. However, it had less ability than the EDTA solution with a statically significant difference ($p < 0.05$). The findings were correlated with Wongklang *et al.* (2014), who studied the antioxidant from ten parts of royal lotus such as petal, stamen, seed, embryo, torus, young leaf, leaf, petal stalk, bud and root. They found that lotus petals have the highest antioxidant ability.

Lastly, the inhibition of lipid peroxidation activity was tested by using the ferric-thiocyanate method to measure the value of LC₅₀. According to the relationship of lipid peroxidation inhibition activity and the concentration (mg/mL) of the bael-rosella mixed herbal drink added with fresh royal lotus petals, it was found that the ability to inhibit lipid peroxidation (LC₅₀) was at 2.73±0.13 mg/mL. Moreover, the ability to inhibit lipid peroxidation (LC₅₀) of the bael-rosella mixed herbal drink added with dried royal lotus petals was at 11.96±1.21 mg/mL. The aforementioned data showed that both the bael-rosella mixed herbal drink added with fresh royal lotus petals and the bael-rosella mixed herbal drink added with dried royal lotus petals contained the ability to inhibit lipid peroxidation (LC₅₀). However, both types of drink had less anti-oxidation activity than vitamin E solution with a statically significant difference ($p < 0.05$).

Therefore, the bael-rosella mixed herbal drink added with dried royal lotus petals showed the highest antioxidant activity. This is because the lotus petals in

the bael-rosella mixed herbal drink had an effect on the anti-oxidation activity.

4. Conclusion

The study of the bael-rosella mixed herbal drink that used 2% of fresh royal lotus petals, or 0.8% of dried royal lotus petals, which had sweetness 10% Brix, obtained the most preference from the consumers. The bael-rosella mixed herbal drink used royal lotus petals has a good appearance, brightness and beautiful colour. It also has good fragrance, good taste and sweetness. In addition, the bael-rosella mixed herbal drink added with dried royal lotus petals had been tested, and the results revealed the highest antioxidant activity. Therefore, consumption of the mixed herbal drink with lotus petals should be promoted as healthy products for health lovers.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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