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## Effects of infusion conditions on bioactive compounds and antioxidant activities of banaba herbal tea

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### Abstract

Banaba (*Lagerstroemia speciosa* L.) is normally consumed as an herbal tea by infusing the banaba powder in hot water. However, the infusion method may influence the bioactive compounds, antioxidant activities, and enzyme inhibitor activities of the tea. Therefore, the objective of this study was to determine the effects of the infusion conditions on the bioactive compounds, antioxidant activities, and on the inhibition against  $\alpha$ -glucosidase and  $\alpha$ -amylase in banaba instant tea. Banaba extract powder (BEP) was produced by water extraction with the ratio of 1:10 and 1:8 (w/w) at 90 °C and was dried using a spray dryer with the addition of 3% gum arabic. Then the effects of the infusion conditions were investigated by varying the water temperatures (80, 90, and 100 °C) and the amount of BEP (2, 4 and 6 g). The investigated parameters were tannin content, total phenolic content (TPC),  $\alpha$ -glucosidase inhibitor,  $\alpha$ -amylase inhibitor and antioxidant activities dinitrosalicylic acid 1,1-diphenyl-2-pyridylhydrazyl (DPPH) scavenging activity and Ferric Reducing Antioxidant Power (FRAP). The results indicated that tannin content, total phenolic content (TPC),  $\alpha$ -glucosidase inhibitor,  $\alpha$ -amylase inhibitor, DPPH and FRAP had significantly increased with increased amounts of BEP ( $p < 0.05$ ). This study also showed that higher water temperature had affected to bioactive compounds and antioxidant activities of the banaba tea ( $p < 0.05$ ). Infusion at 100 °C showed the most effective condition, providing a high concentration of bioactive compounds, antioxidant activities, and  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitor activities. Therefore, tea infused at 100 °C was selected to be mixed with lime juice. The addition of lime juice slightly decreased the bioactive compounds and antioxidant activities of the BEP infusion.

**Keywords:** Antioxidant activity, Banaba, Bioactive compounds, Herbal infusion, Nutritional value

### 1. Introduction

Herbal teas are actually mixtures of several ingredients, such as dried leaves, seeds, grasses, nuts, barks, fruits, flowers, and other botanical elements that give them their taste and provide the benefits of herbal teas. Herbal tea is brewed in the same way as true tea, which is derived from the *Camellia sinensis* plant [1]. Banaba, known scientifically as *Lagerstroemia speciosa* L., is a flowering tree that is native to Southeast Asia. Normally, mature banaba leaves or powders are consumed like herbal teas. The banaba leaves are often used for digestive problems, kidney inflammation, and diabetes [2-3]. The retarding of glucose absorption is done through the inhibition of the carbohydrate-hydrolysing enzymes, such as  $\alpha$ -glucosidase and  $\alpha$ -amylase in the digestive tract [4]. In addition, the banaba leaves contain large amounts of corosolic acid, which has previously been shown to possess anti-diabetic properties [5]. The leaf extract is also shown to possess marked antioxidant activity. Several studies have been conducted for the presence and the activity of antioxidants in tea and herbs, but emphasis has been given to

the herbal infusions of banaba leaves. Tannins are water soluble polyphenol compounds having wide prevalence in banaba leaves [6]. It is a powerful astringent stringent and to some extent, an irritant.

In this study, banaba extract powder (BEP) was prepared to concentrate bioactive compounds and to make it more convenient for infusion. However, the preparation methods of the infusion, such as the amount of solute used, temperature of water, and the addition of other substances may influence the bioactive compounds, antioxidant activity, and inhibition against  $\alpha$ -glucosidase and  $\alpha$ -amylase of the infusion [7]. Lime contains Vitamin C, which has antioxidative properties and can positively influence the antioxidant potential of the infusions [8]. Therefore, the objective of this study was to determine the effects of infusion conditions on the bioactive compounds, antioxidant activities, and the inhibition against  $\alpha$ -glucosidase and  $\alpha$ -amylase of banaba extract powder (BEP) infusion. In addition, the concentration of BEP and the temperature of water used were varied in order to produce the banaba infusion. The best infusion condition with the maximum value of bioactive compounds and antioxidant activity was selected for the purpose of conducting the second part of this study, which covered the effects to the bioactive compounds and antioxidant activity caused by adding lime into the BEP infusion.

## 2. Materials and methods

### 2.1 Raw materials

The dried mature banaba leaves were obtained from Surathanee, Thailand. The leaves were packed in an aluminum foil bag and kept in a dark place at room temperature. Fresh lime fruits were obtained from a local market in Chiang Rai Province.

### 2.2 Chemicals and reagents

Trolox, DPPH (dinitrosalicylic acid 1,1-diphenyl-2-pyridylhydrazyl), TPTZ (2,4,6-tripyridyl-s-triazine),  $\alpha$ -glucosidase, porcine pancreatic  $\alpha$ -amylase, Folin-Ciocalteu reagent, and methanol were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany).

### 2.3 Banaba leaves extraction

Dried leaves of banaba sample were ground using an electric grinder for 10 sec. Then, ground banaba leaves were extracted using deionized water at a ratio of 1:10 (w/w) and 1:8 (w/w) at 90 °C for 30 min and were then filtered.

### 2.4 Preparation of banaba extract powder

The banaba leaf extract was filtered and concentrated by using a vacuum evaporator to give solutions, which contained 10% total solids. Next, the concentrated extract was mixed with 3% gum arabic. These solutions were then spray dried with an inlet air temperature of 160 °C and with an outlet temperature, which was controlled at 100 °C. The dried powders were kept in an aluminum foil bag at 4 °C until needed for further analysis.

### 2.5 The effects of BEP concentrations and temperatures on bioactive compounds and antioxidant activity of BEP infusion

The BEP infusions were prepared by infusing different concentrations of BEP (2, 4, and 6 g) in 150 ml of hot water at three different temperatures (80, 90, and 100 °C). The mixture was stirred with a glass rod for 3 min [9]. The supernatant was then separated by filtering through a filter paper (Whatman no. 42). The infusions were quickly cooled in cold water before being stored at refrigerator temperatures prior to analysis within the same day.

### 2.6 Determination of total tannin content

The tannins were determined by using the Folin-Ciocalteu method [8]. An extract of the sample (0.1 ml) was added to a 10 ml volumetric flask containing 7.5 ml of distilled water and 0.5 ml Folin Phenol reagent. Then 1 ml of 35% sodium carbonate solution ( $\text{Na}_2\text{CO}_3$ ) was added, and the solution was diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. Absorbance for test and standard solutions were measured against the blank at 725 nm with a UV/Visible spectrophotometer. The results of tannins were expressed in terms of gallic acid mg/ml of infusions.

## 2.7 Determination of Total phenolic compound

Total phenol compound (TPC) was determined according to the modified Folin–Ciocalteu method [10]. Distilled water (5 ml) was added to a 10 ml volumetric flask. Approximately 100 µl of each infusion was transferred into the volumetric. Then, 0.2 ml of Folin–Ciocalteu reagent was added and mixed well. After 3 min, 0.4 ml of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution was added, mixed well, and was made up to volume with distilled water. After 1 h reaction in the dark, the absorbance was measured at 725 nm using the spectrophotometer. The results were expressed as mg of gallic acid equivalents (GAE)/ml infusions.

## 2.8 Determination of $\alpha$ -glucosidase inhibition

The Alpha-glucosidase inhibition assay was performed as previously described by Kim et al. [11]. Alpha-glucosidase (50 µl, 0.5 U/ml) and 0.2 M potassium phosphate buffer (pH 6.8, 50 µl) was mixed with 50 µl of test sample. After pre-incubation at 37 °C for 15 min, 3 mM 4-Nitrophenyl  $\beta$ -D-glucopyranoside (pNPG) (100 µl) was added. The enzymatic reaction was allowed to proceed at 37 °C for 10 min and was stopped by the addition of 750 µl of 0.1 M sodium carbonate solution. The enzymatic hydrolysis of the substrate was monitored by the amount of p-nitrophenol, which was released in the reaction mixture at 400 nm. Acarbose (1 mg/ml) was used as a positive control. The inhibitory activity of  $\alpha$ -glucosidase was assessed by the following equation:

$$\% \text{ inhibitory of } \alpha\text{-glucosidase} = (\text{Abs blank} - (\text{Abs sample} - \text{Abs background})) / (\text{Abs blank}) \times 100 \quad (1)$$

## 2.9 Determination of $\alpha$ -amylase inhibition

The  $\alpha$ -amylase inhibitory assay of banana leaf extracts were evaluated according to a previously described method by Ranilla et al. [12]. In brief, 0.5 ml of BEP infusion was mixed with 0.5 ml of  $\alpha$ -amylase solution (0.5 mg/ml) and with 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The mixture was incubated at room temperature for 10 min and 0.5 ml of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added. The resulting mixture was incubated at room temperature for 10 min, and the reaction was terminated by using 1 ml of di-nitrosalicylic acid color reagent. At this time, the test tubes were placed in a water bath (100 °C and 5 min) and cooled until room temperature was reached. The mixture was then diluted with 10 ml of deionized water, and then the absorbance was determined at 540 nm. The inhibition of  $\alpha$ -amylase was calculated using the following equation:

$$\% \text{ inhibition of } \alpha\text{-amylase} = (\text{Abs control} - \text{Abs sample}) / (\text{Abs control}) \times 100 \quad (2)$$

In which Abs control corresponds to the absorbance of the solution without the sample (buffer instead of extract) and with  $\alpha$ -amylase solution and the Abs sample corresponds to the solution with sample and the  $\alpha$ -amylase solution.

## 2.10 Determination of DPPH Free Radical Scavenging assay

The scavenging effects of the infusion of BEP against DPPH radicals were determined according to a method by Atoui et al. [13]. Approximately 100 µl of each BEP infusion was mixed with 2.9 ml of methanolic DPPH reagent solution. The mixture was vortexed for 10 sec and allowed to stand for 30 min in a dark place. Then the absorbance was measured at 515 nm using a spectrophotometer (Uv-Vis spectrophotometer, Thermo Scientific, Genesys 20). Radical scavenging activity was calculated using the following equation:

$$\% \text{ inhibition of DPPH} = (\text{Abs control} - \text{Abs sample}) / (\text{Abs control}) \times 100 \quad (3)$$

In which the absorbance of the solution without sample (DPPH only) is expressed as Abs control and the solution, combined with the sample, is expressed by Abs sample.

## 2.11 Determination of Ferric Reducing Antioxidant Power (FRAP) assay

This assay is based on research by Thaipong et al. [14]. Briefly, a sample infusion (100 µl) was reacted with 3 ml of the FRAP solution for 30 min under dark conditions. The reading of the coloured product (ferrous tripyridyltriazine complex) was measured at 593 nm using a UV-VIS spectrophotometer. The calibration curve was plotted between the ferrous sulfate concentration (µM) and the absorbance at 595 nm. The Fe (II) reducing activity was determined as µmole equivalents of ferrous sulfate/ml infusions.

## 2.12 Effects of adding lime juice on bioactive compounds and antioxidant activity of BEP infusion

BEP (6 g) was infused in 150 ml of hot water with the amount of BEP and the temperature of hot water at 100 °C. The mixture was stirred with a glass rod for 3 min. Then, the infusion was separated by filtering the mixture through a Whatman no. 42 filter paper [9]. Different amounts of freshly squeeze lime juice (0, 1, 2, 3, and 4 ml) were added to the infusion and then carefully stirred. The bioactive compounds and antioxidant activity of the BEP infusion were determined as previously mentioned. In addition, the pH of the mixture was determined by using a pH meter (HI211, HANNA Instrument, USA).

## 2.13 Statistical analysis

All the measurements were performed in triplicate, and the results were expressed as mean $\pm$ SD. Statistical analysis was carried out by using the SPSS package (SPSS 16.0 for Windows, SPSS Inc, Chicago, IL). When ANOVA identified differences among groups, the differences in mean values were compared using Tukey's HSD test at the level of significance of 5% ( $P < 0.05$ ).

## 3. Results and discussion

### 3.1 The effects of BEP concentrations and temperatures on bioactive compounds and antioxidant activities of BEP infusions

Table 1 shows that the tannin content in the sample had significantly increased when 100 °C hot water was used as compared to that of 80 °C ( $P < 0.05$ ). In addition, increasing the BEP concentration also significantly increased the tannin content ( $P < 0.05$ ). Furthermore, the tannin content with the ratio of 1:10 was found to be significantly higher than that of 1:8 ( $P < 0.05$ ). The banaba extract with a ratio of 1:10 showed high total phenolic contents as compared to that of 1:8 ( $p < 0.05$ ). In addition, increasing the water temperature had significantly increased the tannin contents ( $p < 0.05$ ).

Table 1 also reveals that increasing of infusion temperature had significant increased the DPPH and FRAP values, whereas the infusion with 1:10 ratio also increased these antioxidant activities ( $p < 0.05$ ). The results also showed that the extraction of banaba leave with ratio 1:10 had been more efficient in releasing bioactive compounds and antioxidant activity to the infusions than the extraction with a ratio 1:8.

**Table 1** The bioactive compounds and antioxidant activity of BEP infusions as determined by tannin content, total polyphenol content, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reduction activity potential (FRAP) assays from different extraction ratios of the banaba powder.

Amount of BEP (g)	Temp of water (°C)	Tannin (mg GAE/ml)		TPC (mg GAE/ml)		DPPH ( $\mu$ mol Trolox/ml)		FRAP ( $\mu$ mol ferrous sulfate/ml)	
		1:10	1:8	1:10	1:8	1:10	1:8	1:10	1:8
2	80	2.30 $\pm$ 0.01 <sup>bA</sup>	2.20 $\pm$ 0.06 <sup>bB</sup>	1.93 $\pm$ 0.04 <sup>cA</sup>	1.10 $\pm$ 0.02 <sup>aB</sup>	30.67 $\pm$ 0.37 <sup>bA</sup>	26.85 $\pm$ 1.40 <sup>cB</sup>	31.06 $\pm$ 0.51 <sup>cA</sup>	26.82 $\pm$ 0.60 <sup>cB</sup>
	90	2.38 $\pm$ 0.01 <sup>aA</sup>	2.28 $\pm$ 0.03 <sup>abB</sup>	2.00 $\pm$ 0.02 <sup>bA</sup>	1.14 $\pm$ 0.03 <sup>aB</sup>	32.05 $\pm$ 1.44 <sup>bA</sup>	30.89 $\pm$ 0.46 <sup>bB</sup>	43.03 $\pm$ 0.18 <sup>bA</sup>	30.25 $\pm$ 0.60 <sup>bB</sup>
	100	2.40 $\pm$ 0.03 <sup>aA</sup>	2.35 $\pm$ 0.03 <sup>aB</sup>	2.08 $\pm$ 0.02 <sup>aA</sup>	1.15 $\pm$ 0.03 <sup>aB</sup>	35.95 $\pm$ 0.70 <sup>aA</sup>	33.14 $\pm$ 0.05 <sup>aB</sup>	45.87 $\pm$ 1.50 <sup>aA</sup>	32.03 $\pm$ 0.52 <sup>aB</sup>
4	80	2.87 $\pm$ 0.06 <sup>aA</sup>	2.42 $\pm$ 0.03 <sup>bB</sup>	2.42 $\pm$ 0.04 <sup>aA</sup>	2.15 $\pm$ 0.01 <sup>aB</sup>	58.39 $\pm$ 0.79 <sup>bA</sup>	40.34 $\pm$ 2.92 <sup>bB</sup>	61.31 $\pm$ 0.55 <sup>cA</sup>	47.91 $\pm$ 3.60 <sup>bB</sup>
	90	2.90 $\pm$ 0.03 <sup>aA</sup>	2.50 $\pm$ 0.02 <sup>aB</sup>	2.44 $\pm$ 0.02 <sup>aA</sup>	2.15 $\pm$ 0.10 <sup>aB</sup>	58.11 $\pm$ 1.78 <sup>bA</sup>	49.34 $\pm$ 0.95 <sup>aB</sup>	74.37 $\pm$ 9.71 <sup>bA</sup>	49.99 $\pm$ 0.28 <sup>bB</sup>
	100	2.93 $\pm$ 0.01 <sup>aA</sup>	2.52 $\pm$ 0.02 <sup>aB</sup>	2.47 $\pm$ 0.02 <sup>aA</sup>	2.21 $\pm$ 0.03 <sup>aB</sup>	65.63 $\pm$ 0.80 <sup>aA</sup>	52.64 $\pm$ 1.08 <sup>aB</sup>	96.68 $\pm$ 0.63 <sup>aA</sup>	58.05 $\pm$ 0.86 <sup>aB</sup>
6	80	3.03 $\pm$ 0.00 <sup>aA</sup>	2.64 $\pm$ 0.17 <sup>bB</sup>	2.54 $\pm$ 0.01 <sup>aA</sup>	2.22 $\pm$ 0.07 <sup>aB</sup>	68.03 $\pm$ 0.80 <sup>cA</sup>	55.85 $\pm$ 0.49 <sup>cB</sup>	125.58 $\pm$ 1.78 <sup>cA</sup>	80.30 $\pm$ 0.92 <sup>cB</sup>
	90	3.04 $\pm$ 0.02 <sup>aA</sup>	2.74 $\pm$ 0.06 <sup>abB</sup>	2.55 $\pm$ 0.01 <sup>aA</sup>	2.24 $\pm$ 0.03 <sup>aB</sup>	72.83 $\pm$ 0.21 <sup>bA</sup>	56.88 $\pm$ 0.09 <sup>bB</sup>	143.98 $\pm$ 1.83 <sup>bA</sup>	86.56 $\pm$ 1.26 <sup>bB</sup>
	100	3.06 $\pm$ 0.03 <sup>aA</sup>	2.92 $\pm$ 0.04 <sup>aB</sup>	2.58 $\pm$ 0.05 <sup>aA</sup>	2.28 $\pm$ 0.02 <sup>aB</sup>	74.65 $\pm$ 0.57 <sup>aA</sup>	58.03 $\pm$ 0.33 <sup>aB</sup>	166.18 $\pm$ 2.45 <sup>aA</sup>	100.42 $\pm$ 0.95 <sup>aB</sup>

Values represent mean  $\pm$  SD ( $n = 3$ ). Different lowercase letters in the same column indicate a significant difference between temperatures, whereas different uppercase letters in the same row indicate significant differences between the ratios within the constant weight of powder ( $p < 0.05$ ).

### 3.2 Effects of BEP concentrations and temperatures on $\alpha$ -glucosidase and $\alpha$ -amylase inhibitor activities

Table 2 shows that the infusion of 6 g banaba extract powder at 100 °C had exhibited the highest inhibitory activities on  $\alpha$ -amylase and  $\alpha$ -glucosidase as compared to the other samples ( $p < 0.05$ ). In addition, acarbose was found to be significantly lower in  $\alpha$ -glucosidase inhibitory activities than the banaba extract powder ( $p < 0.05$ ). The  $\alpha$ -glucosidase inhibitory activities ranged from 78.77% to 87.17% for the 1:10 BEP infusion, while for the 1:8 BEP infusion, the  $\alpha$ -glucosidase inhibitory activities ranged from 70.36% to 82.50%. Their inhibitory activity was high even in the infusion with a lower amount of powder. The results showed that the temperature of water (80, 90, or 100 °C) had not significantly influenced the activity of the  $\alpha$ -glucosidase inhibitor in almost all infusions ( $p > 0.05$ ). In the case of  $\alpha$ -amylase inhibition potential, lower inhibition was exhibited as compared to

the  $\alpha$ -glucosidase inhibitor. The  $\alpha$ -amylase inhibition activity ranged from 58.94% to 75.94% and from 26.41% to 66.19% for 1:10 and 1:8 BEP infusions, respectively.

**Table 2** Enzyme inhibitor activity of the BEP infusions from different extraction ratios of banaba powder.

Amount of BEP (g)	Temp of water (°C)	$\alpha$ -glucosidase inhibitor activity (%)		$\alpha$ -amylase inhibitor activity (%)	
		1:10	1:8	1:10	1:8
2	80	78.77 $\pm$ 0.56 <sup>aA</sup>	70.36 $\pm$ 0.34 <sup>aB</sup>	58.94 $\pm$ 0.61 <sup>bA</sup>	26.41 $\pm$ 3.46 <sup>aB</sup>
	90	79.69 $\pm$ 1.08 <sup>aA</sup>	70.78 $\pm$ 2.11 <sup>aB</sup>	64.97 $\pm$ 1.00 <sup>aA</sup>	27.69 $\pm$ 4.63 <sup>aB</sup>
	100	78.96 $\pm$ 3.22 <sup>aA</sup>	71.35 $\pm$ 1.31 <sup>aB</sup>	65.43 $\pm$ 2.05 <sup>aA</sup>	29.54 $\pm$ 1.29 <sup>aB</sup>
4	80	82.83 $\pm$ 1.28 <sup>aA</sup>	75.10 $\pm$ 0.75 <sup>aB</sup>	70.30 $\pm$ 0.52 <sup>aA</sup>	43.39 $\pm$ 0.29 <sup>bB</sup>
	90	83.10 $\pm$ 0.72 <sup>aA</sup>	75.42 $\pm$ 1.35 <sup>aB</sup>	70.55 $\pm$ 0.42 <sup>aA</sup>	45.43 $\pm$ 2.79 <sup>bB</sup>
	100	83.45 $\pm$ 0.50 <sup>aA</sup>	76.37 $\pm$ 0.95 <sup>aB</sup>	71.06 $\pm$ 1.91 <sup>aA</sup>	49.79 $\pm$ 2.20 <sup>aB</sup>
6	80	86.85 $\pm$ 0.76 <sup>aA</sup>	79.62 $\pm$ 1.60 <sup>bB</sup>	73.66 $\pm$ 0.72 <sup>bA</sup>	62.14 $\pm$ 3.06 <sup>aB</sup>
	90	85.85 $\pm$ 0.31 <sup>bA</sup>	80.28 $\pm$ 0.68 <sup>abB</sup>	74.51 $\pm$ 0.26 <sup>abA</sup>	64.38 $\pm$ 3.53 <sup>bB</sup>
	100	87.17 $\pm$ 1.04 <sup>aA</sup>	82.50 $\pm$ 0.86 <sup>aB</sup>	75.74 $\pm$ 1.38 <sup>aA</sup>	66.19 $\pm$ 1.92 <sup>aB</sup>
Acarbose	100	84.26 $\pm$ 0.24 <sup>bA</sup>	77.26 $\pm$ 0.54 <sup>cB</sup>	-	-

Values represent mean  $\pm$  SD (n=3). Different lowercase letters in the same column indicate a significant difference between temperatures, whereas different uppercase letters in the same row indicate a significant difference between ratios within constant weight of powder (p<0.05).

### 3.3 The effects of adding lemon juice to the bioactive compounds and antioxidant activities of banaba infusions

In this study, the effects of adding lemon juice on the bioactive compounds and the antioxidant activities of banaba infusions was tested (Table 3). The pH values of several commercial brands of lemon tea were measured, and the pH was found to be in the range of 2.95-3.05. Therefore, the amount of lemon juice, which was added, was for the purpose of reaching the studied pH. Based on this study, the addition of lemon juice to the infusions exhibited a significant effect on their antioxidant activities (p<0.05). In the infusions of 1:10 banaba extract powder, the bioactive compounds, and antioxidant activities were slightly decreased after 1 ml to 4 ml of lemon juice had been added to 15 ml infusions. In the infusions of 1:8 banaba extract powder, a significant effect was not shown on the TPC, DPPH, and FRAP values after the lemon juice had been added (p>0.05). Yet, the results indicated that there had been significant decrease in the tannin content of the infusions (p<0.05).

**Table 3** Bioactive compounds and antioxidant activity of banaba infusions after the addition of lemon juice.

Extraction ratio of banaba leaves	Amount of lemon juice added (ml)	pH	Tannins (mg GAE/ml)	TPC (mg GAE/ml)	DPPH ( $\mu$ mol Trolox/ml)	FRAP ( $\mu$ mol ferrous sulfate/ml)
1:10	0	4.66 $\pm$ 0.02 <sup>a</sup>	2.88 $\pm$ 0.05 <sup>a</sup>	2.90 $\pm$ 0.06 <sup>a</sup>	83.63 $\pm$ 1.18 <sup>a</sup>	94.45 $\pm$ 0.83 <sup>a</sup>
	1	3.47 $\pm$ 0.01 <sup>b</sup>	2.86 $\pm$ 0.02 <sup>ab</sup>	2.83 $\pm$ 0.04 <sup>ab</sup>	81.25 $\pm$ 0.70 <sup>ab</sup>	92.96 $\pm$ 0.84 <sup>a</sup>
	2	3.14 $\pm$ 0.02 <sup>c</sup>	2.85 $\pm$ 0.01 <sup>abc</sup>	2.76 $\pm$ 0.05 <sup>bc</sup>	79.59 $\pm$ 2.71 <sup>bc</sup>	93.55 $\pm$ 0.94 <sup>a</sup>
	3	3.01 $\pm$ 0.03 <sup>d</sup>	2.80 $\pm$ 0.02 <sup>c</sup>	2.69 $\pm$ 0.03 <sup>cd</sup>	77.88 $\pm$ 2.20 <sup>bc</sup>	91.25 $\pm$ 0.57 <sup>b</sup>
	4	2.92 $\pm$ 0.01 <sup>e</sup>	2.83 $\pm$ 0.02 <sup>bc</sup>	2.62 $\pm$ 0.04 <sup>d</sup>	76.00 $\pm$ 2.11 <sup>c</sup>	89.02 $\pm$ 0.91 <sup>c</sup>
1:8	0	4.71 $\pm$ 0.01 <sup>a</sup>	2.86 $\pm$ 0.02 <sup>a</sup>	2.52 $\pm$ 0.27 <sup>a</sup>	60.64 $\pm$ 0.13 <sup>a</sup>	90.08 $\pm$ 2.57 <sup>a</sup>
	1	3.48 $\pm$ 0.03 <sup>b</sup>	2.85 $\pm$ 0.01 <sup>ab</sup>	2.50 $\pm$ 0.36 <sup>a</sup>	59.73 $\pm$ 1.77 <sup>a</sup>	88.86 $\pm$ 1.62 <sup>a</sup>
	2	3.15 $\pm$ 0.01 <sup>c</sup>	2.83 $\pm$ 0.01 <sup>b</sup>	2.44 $\pm$ 0.30 <sup>a</sup>	60.48 $\pm$ 1.18 <sup>a</sup>	85.49 $\pm$ 6.14 <sup>a</sup>
	3	2.99 $\pm$ 0.01 <sup>d</sup>	2.78 $\pm$ 0.01 <sup>c</sup>	2.44 $\pm$ 0.23 <sup>a</sup>	57.55 $\pm$ 0.89 <sup>b</sup>	86.24 $\pm$ 0.28 <sup>a</sup>
	4	2.90 $\pm$ 0.02 <sup>e</sup>	2.77 $\pm$ 0.04 <sup>c</sup>	2.38 $\pm$ 0.21 <sup>a</sup>	56.91 $\pm$ 1.10 <sup>b</sup>	87.95 $\pm$ 4.70 <sup>a</sup>

Values represent mean  $\pm$  SD (n=3). Different lowercase letters in the same column indicate a significant difference. (p<0.05).

## 4. Discussion

### 4.1 The effects of BEP concentrations and temperatures on bioactive compounds and antioxidant activity of BEP infusion

The present results are similar to the findings from Rehman et al. [15], who found that increasing the water temperature from 90 to 100 °C had caused increases in tannins in commercial brands of teas. Furthermore, the tannin content with the ratio of 1:10 was significantly higher than that of 1:8 (P<0.05). The tannins may have increased due to increases in the extraction efficiency, which can occur when the ratio of material to solvent is increased. Perva-Uzunalić et al. [16] also reported that extraction of green tea leaves with water at a ratio of 1 g to 40 ml yielded lower bioactive compounds as compared to that of 1 g to 100 ml ratio (p<0.05).

According to a previous study, our results confirmed that the total phenolic content of herbal infusions (linden and chamomile) had been affected by the water temperatures (60, 80 and 100 °C), which indicated that water at higher temperatures had extracted higher TPC [9]. In addition, Table 1 reveals that increasing the infusion temperature had significantly increased the DPPH and FRAP values, whereas the infusion with 1:10 ratio had also increased these antioxidant activities ( $p < 0.05$ ). Regarding the extraction efficiency of antioxidants, the results obtained were due to the effects of temperature and the ratio of material to solvent. The obtained results suggested that higher water temperatures and higher amounts of banaba extract powder are the best combination for the extraction of bioactive compounds of banaba extract. Some previously conducted studies have shown that there is a dependence between phenolic contents and antioxidant activity to temperature. Horžić et al. [9] observed that the antioxidant activities of herbal extracts had increased with water temperature ( $100\text{ °C} > 80\text{ °C} > 60\text{ °C}$ ). In addition, Astill et al. [17] stated that the soluble solids content extracted after a given time period is directly proportional to the leaf to water ratio of the tea, which supports our results by showing that when a higher amount of banaba extract powder is used, the process yields higher bioactive compounds and greater antioxidant activities. The results also showed that the extraction of banaba leaves with a ratio 1:10 had been more efficient in releasing bioactive compounds and antioxidant activity to the infusions than the extraction with a ratio 1:8.

#### 4.2 The effects of BEP concentrations and temperatures on $\alpha$ -glucosidase and $\alpha$ -amylase inhibitor activities

Type 2 diabetes involves a rapid increase in blood glucose levels due to hydrolysis of starch by pancreatic  $\alpha$ -amylase and the absorption of glucose in the small intestine by  $\alpha$ -glucosidase [12]. The inhibition of these two enzymes may be one of the most effective approaches to controlling Type 2 diabetes mellitus. Inhibition of the enzymes will delay carbohydrate digestion and thus, prolong the overall carbohydrate digestion time. It will result in a reduction of the rate of glucose absorption and will cause blunting to the post-prandial plasma glucose rise [4]. The  $\alpha$ -glucosidase inhibitors inhibit enzymes in the intestine. They are effective in delaying glucose absorption and preventing elevation of the post-prandial blood glucose level. Therefore, they play a significant role as chemotherapeutic agents for non-insulin-dependent diabetes mellitus [18]. This study may indicate that BEP infusion has an interesting functionality in the potential control of glucose absorption and is likely not to generate high effect on  $\alpha$ -amylase inhibitory activity [19].

#### 4.3 The effects of adding lemon to the bioactive compounds and antioxidant of banaba infusions

The addition of lemon juice to herbal teas provides a means of lowering the astringency of the herbal extracts [9]. Langley-Evans [20] indicated that phenolic compounds interact with the lipid fraction from the lemon juice, and could, thus, lower the antioxidant activity or perhaps, produce no change at all. The study by Horžić et al. [9] showed the same trends with the results from this study. The addition of lemon juice to tea extracts was not shown to have significant effects on their antioxidant activities.

### 5. Conclusions

The study showed that the preparation variables had greatly influenced the composition of the infusions. The amount of powder and the water temperature, as well as the addition of lemon juice, has been shown to have had an impact on the BEP infusions, which, in turn, caused significant changes to the bioactive compounds, the antioxidant activities, and enzyme inhibitor activities in the infusions. The infusion at 100 °C showed the most effective condition by providing a high concentration of bioactive compounds, antioxidant activities, and  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitor activities. The results suggest that the most suitable condition for producing maximum value of bioactive compounds and antioxidant activity for banaba infusion is by infusing 6 g of BEP in boiling water. Furthermore, it was found that the addition of lemon juice had caused a significant decrease in the bioactive compounds and antioxidant activities of the banaba infusions.

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### 7. References

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