

Antioxidant activity and α -amylase inhibitory of herbal drink from the combination of *Blumea balsamifera* L and *Coccinia grandis* L

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Abstract

The leaves of *Blumea balsamifera* L and *Coccinia grandis* L are rich in flavonoids and showed a potential role as an α -amylase inhibitor with antioxidant activity. This study aimed to evaluate the total flavonoid content, total phenolic, antioxidant activity, and α -amylase inhibitor activity of combination drinks derived from the leaves of *B. balsamifera* L and *C. grandis* L. The combined drink was extracted by boiling the dried of the two leaves in 200 mL of distilled water for 5 mins with various ratios. Analysis of total flavonoid and total phenol content was carried out using $AlCl_3$ and Folin-Ciocalteu methods. Determination of antioxidant activity and α -amylase inhibitory activity was carried out using the DPPH method and iodine-starch test. The ratios of *B. balsamifera* L to *C. grandis* L. (1:3, BC13) showed the highest TFC of 313.04 ± 5.75 mg QE/g dry matter. Furthermore, the ratios of *B. balsamifera* L to *C. grandis* L. (1:3 and 1:1) showed the highest TPC of 107.66 ± 5.50 and 110.66 ± 5.50 mg GAE/g dry matter, respectively. BC13 had the highest antioxidant activity and antidiabetic activity with IC_{50} values of 1.758 ± 0.133 mg/mL and 1.109 ± 0.067 mg/mL, respectively. It can be concluded that the combination of *B. balsamifera* L and *C. grandis* L could be used as a source of flavonoids and phenolic compounds, with potential antioxidant and anti-diabetic agents.

1. Introduction

For decades, the traditional herbal drink has played an important role in the treatment of diabetes mellitus (DM). The absorption of glucose in the intestine is facilitated by α -amylase and the inhibition of this enzyme is currently being introduced in the management of DM type 2 (Oyedemi *et al.*, 2017). The action of α -amylase inhibitor results in the reduction of starch hydrolysis, which is essential for the regulation of blood sugar levels in diabetic patients (Bhat *et al.*, 2011; Sy *et al.*, 2017; Thengyai *et al.*, 2019). Several hypoglycemic drugs have been extensively used to treat diabetic patients (Arumugam *et al.*, 2013), such as sulphonylureas, biguanide, glinide (Mohammed *et al.*, 2016), metformin, and thiazolidinedione (Meenatchi *et al.*, 2017). Unfortunately, those drugs have side effects such as diarrhoea, flatulence, and nausea (Hollander, 1992).

The potential role of herbal as α -amylase inhibitor has been studied and reviewed. A number of bioactive compounds such as glycosides, alkaloids, galactomannan

gum, polysaccharides, hypoglycans, peptidoglycans, guanidine, steroids, glycopeptides, and terpenoids that are present in herbal plants play an important role against hyperglycemia conditions. The intake of herbal-rich food/drink could be used as a strong α -amylase inhibitor (Soud *et al.*, 2004). Therefore, it is necessary to develop a herbal-rich drink as a strong α -amylase inhibitor. Recently, the development of healthy drinks based on herbal plants is increasing due to minimum side effects (Modak *et al.*, 2007). Therefore, traditional herbal drinks are of interest to be developed as an alternative treatment for diabetes (Thengyai *et al.*, 2019). In addition, high scavenging reactions of herbal plants to reactive oxygen species (ROS) could be useful for diabetic patients (Arumugam *et al.*, 2013; Mamun-or-Rashid *et al.*, 2014). Herbal plants contained a wide variety of antioxidant compounds such as ascorbic acid, flavonoid, phenolic, carotenoids, and tocopherol, which differ in their mechanisms of action and compositions.

Blumea balsamifera L has possessed physiological effects such as hypoglycemic effect, treating various

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digestive disorders such as diarrhoea, dysentery, flatulence, and dyspepsia (Roy *et al.*, 2013). *Blumea balsamifera* L contains polyphenol compounds including xanthoxylin, eugenol, and dimethoxydurene, flavonoids, dihydroflavons (Pang *et al.*, 2014), terpene, tannins, flavonoids, blumeatin, and quercetin (Roy *et al.*, 2013). Quercetin has been shown as antidiabetic activity against streptozotocin-induced diabetes in experimental rats (Najafian, 2015). Another plant such as *C. grandis* L. also shows the potential activity as an α -amylase inhibitor. Utilization of the roots, leaves, and fruit of *C. grandis* L has been studied as a useful herbal drink to treat wound healing, ulcers, jaundice, and diabetes mellitus. *C. grandis* leaves have shown hypoglycemic properties and antioxidants activity (Mohammed *et al.*, 2016). *Coccinia grandis* L contains high metabolic secondary compounds such as phenolic compounds, alkaloids, saponins, flavonoids, glycosides, xyloglucan, taraxerol, carotenoids, and gytioxanthin. Saponin can provide high antidiabetic activity, and has inhibitory activity against α -amylase and α -glucosidase in rats with diabetes mellitus type 2 (Waisundara *et al.*, 2015). Although *C. grandis* L has been proven as an antidiabetic agent, its efficacy is still low compared to synthetic drugs. Therefore, combination with other herbal plants that show the potential effect as an antidiabetic agent is required (Attanayake *et al.*, 2016).

Traditional herbal drinks are commonly made based on a combination of various types of plants. The purpose of this combination is to enhance the pharmacological properties of the individual and to reduce its toxicity, as an example is a combination of green tea and sea Lavender (*Limonium angustifolia*) as an antidiabetic and stress oxidative scavenging against ROS (Rodrigues *et al.*, 2019), and drinks from a combination of four components (pineapple, carrot, orange, and juice *Hibiscus sabdariffa* or Rosella) (Ogundele *et al.*, 2016). The combination of *B. balsamifera* L and *C. grandis* L has never been studied, therefore the study aims to study the combination of these two plants as α -amylase inhibitors with high antioxidant activity.

2. Materials and methods

2.1 Materials

Blumea balsamifera L and *C. grandis* L leaves were harvested during January and February 2019 in Badung, Bali Province, and Bondowoso, East Java, Indonesia, respectively. The materials used in this studied were *B. balsamifera* L leaf powder, *C. grandis* L leaf powder, distilled water, ethanol (Merck, Germany), methanol (Merck, Germany), aluminium chloride (Merck, Germany), potassium acetate (Merck, Germany), Folin-Ciocalteu's reagents (Merck, Germany), potassium

phosphate (Merck, Germany), Error acid (Sigma-Aldrich), DPPH (Sigma-Aldrich), α -amylase (Sigma-Aldrich), iodine (Sigma-Aldrich), p-nitrophenyl- α -D-glucopyranoside (Sigma-Aldrich), Acarbose, Na_2CO_3 (Sigma-Aldrich). All reagents were of analytical grade.

2.2 Preparation of herbal drink

Sample preparation was performed according to Rodrigues *et al.* (2019). In this study fresh *B. balsamifera* L and *C. grandis* L leaves were washed and air-dried for 24 hours and followed by drying for 3 days at 50°C. The dried leaves were ground to a powder using a blender (Philips). Then the leaf powder is sifted using the 60 sieves and mesh 100. Subsequently, the powder obtained from 100 mesh sieves was stored at 4°C prior to analysis. A combination of herbal drinks was prepared based on Rodrigues *et al.* (2019) with slight modification. The various ratio of *B. balsamifera* L (BB) and *C. grandis* L (CG) was prepared. The combination ratios of *B. balsamifera* L and *C. grandis* L were 1:3 (BC13), 1:1 (BC11), 3:1 (BC31). Approximately 200 mL of distilled water was added to the powder and boiled for 5 mins. All of the samples were filtered through Whatman No. 41 paper.

2.3 Total flavonoid content

The total flavonoid content was determined, according to the colourimetric aluminium chloride (AlCl_3) by using the procedure described by Attanayake *et al.* (2016). Briefly, 0.50 mL of the samples extract was transferred into the test tube, and 1.5 mL of 95% ethanol, 0.10 mL of 10% aluminium chloride, 0.10 mL of potassium acetate 1 M, and 2.8 mL of distilled water. The mixed solution was incubated at 27°C for 30 mins. After incubation, the absorbance was read at 415 nm using a UV-vis spectrophotometer (Genesys 10S). The absorbance value was then converted to TFC content and was expressed in milligrams equivalent of quercetin (QE)/g dry weight using a standard curve using various concentrations of quercetin (0-50 $\mu\text{g}/\text{mL}$).

2.4 Total phenolic content

A method described by Bi *et al.* (2016) was used to evaluate the amount of TPC of the samples, using Folin Ciocalteu and gallic acids as standard. Briefly, 100 μL of the sample was transferred into a test tube, and 200 μL of Folin-Ciocalteu reagent and 800 μL of Na_2CO_3 700 nM was added to the reaction mixture and incubated for 2 hrs in the dark at room temperature. After 2 hrs of incubation, the absorbance was read at 765 nm using a UV-vis spectrophotometer. The absorbance values were then converted to TPC content and were expressed in milligrams equivalent of gallic acid (GAE)/g dry weight using a standard curve using various concentrations of

gallic acid (0-100 µg/mL).

2.5 DPPH radical scavenging activity

The total antioxidant activity (TA) of samples based on DPPH free radical scavenging was determined by using the method described by Neagu *et al.* (2018). The total antioxidant potential of the samples was evaluated in terms of the radical scavenging ability of the extract using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. 200 µL of samples were added with 3.5 mL of DPPH in methanol and incubated for 60 mins in the darkroom. The absorbances were read at 517 nm wavelength.

$$\text{Radical scavenging activity (\%)} = \frac{A_B - A_A}{A_B} \times 100$$

Where AB: control absorbance and AA: sample absorbance

2.6 α-amylase inhibition assay

The α-amylase inhibitory activity was performed using the iodine-starch test (Neagu *et al.*, 2018). A total of 30 µL extracts in methanol solution (1.0 mg/mL) were added with 100 µL of starch substrate in phosphate buffer (0.25 M, pH 7.0). After 30 mins of incubation at 37°C, 10 µL of α-amylase (50 µg/mL in phosphate buffer, pH 7.0) and 10 µL 0.25 M phosphate buffer (pH 7.0) was added to the mixture. Subsequently, 100 µL of iodine solution 0.01 N and 500 µL distilled water was added to the mixtures. The absorbances were read at 660 nm wavelength.

$$\text{Inhibition (\%)} = \frac{(A - B)}{A} \times 100$$

Where A: sample absorbance and B: control absorbance

2.7 Statistical analysis

All the analyses were performed in triplicate. The results were expressed as mean ± SD. The comparisons between or among test groups were determined by one-way analysis of variance (one-way ANOVA) with Tukey methods range test using SPSS statistic 24 software. ANOVA data with a 95% confidence level was considered statistically significant. The correlation

between total flavonoid, total phenolic compound content, antioxidant activities, and α-amylase activities were determined using by Pearson test.

3. Results

3.1 Total flavonoid

The TFC content of combination herbal drinks of *B. balsamifera* L and *C. grandis* L was determined using the AlCl₃ method. A combination of *B. balsamifera* L and *C. grandis* L in various ratios was 175.90±16.54 to 313.04±5.75 mg QE/g dry weight (Table 1). The extract of *B. balsamifera* L and *C. grandis* L showed TFC content between 145.47±3.71 to 261.95±35.93 mg QE/g dry weight and 211.45±0.01 to 375.72±4.90 mg QE/g dry weight, respectively. The combination of *B. balsamifera* L and *C. grandis* L drinks in different concentrations results in different TFC. The combination of *B. balsamifera* L and *C. grandis* L in ratio 3:1 at concentrations of 0.5 g resulted in the highest TFC, while *B. balsamifera* L and *C. grandis* L in ratio 1:3 at concentrations of 1.5 g resulted in the lowest TFC.

3.2 Total Phenolic

Phenolic is a natural component that is found in large quantities and has a role as an antioxidant (Ahangarpour *et al.*, 2019). The combination drink of *B. balsamifera* L and *C. grandis* L produces a total phenolic of 28,47±1.12 to 110,66±5.50 mg of GAE/g dry material (Table 2). The *B. balsamifera* L leaf extract has a TPC was 49.00±0.001 to 112.33±5.68 mg GAE/g dry material, while the *C. grandis* L leaf extract has a TPC was 15.33±1.15 to 54.33±2.5 mg of GAE/g dry material. The combination of *B. balsamifera* L and *C. grandis* L in ratio 1:1 and also *B. balsamifera* L and *C. grandis* L in ratio 3:1 at concentrations of 0.5 g resulted in the highest TPC, while the combination of *B. balsamifera* L and *C. grandis* L in 1:3 drink at 2 g concentrations resulted in the lowest TPC.

3.3 Antioxidant activity

The combination drinks of *B. balsamifera* L and *C. grandis* L produce antioxidant activities were 12.34±0.96

Table 1. Total flavonoid content of combination herbal drink

Sample	TFC (mg QE/g dry weight)				
	0.5	1	1.5	2	2.5
BB	375.72±4.90 ^m	290.40±17.94 ^{kl}	280.31±1.71 ^k	247.64±0.95 ^{ij}	211.74±3.08 ^{fgh}
BC31	313.04±5.75 ^l	204.71±16.25 ^{efgh}	210.99±1.37 ^{fgh}	234.69±5.31 ^{hi}	181.52±1.72 ^{def}
BC11	219.20±1.25 ^{ghi}	175.18±7.34 ^{bcd}	159.18±4.32 ^{bc}	227.62±2.99 ^{hi}	179.99±0.94 ^{def}
BC13	203.62±3.49 ^{efgh}	175.90±16.54 ^{bcd}	122.46±6.60 ^a	192.66±4.01 ^{defg}	188.55±3.70 ^{def}
CG	261.95±35.93 ^j	169.38±10.54 ^{bcd}	169.08±2.62 ^{bcd}	145.47±3.71 ^{ab}	165.72±1.82 ^{bcd}

Values are presented as mean±standard deviation. Values with different superscript within the same column are significantly different (p<0.05).

Table 2. Total phenolic content of combination herbal drink

Sample	TPC (mg GAE/g dry weight)				
	0.5	1	1.5	2	2.5
BB	112.33±5.68 ^k	73.66±1.15 ^j	74.66±0.57 ^j	53.66±0.57 ^{gh}	49.00±0.00 ^{fg}
BC31	107.66±5.50 ^k	57.33±1.52 ^{hi}	44.66±1.52 ^{ef}	50.33±0.57 ^{fg}	43.19±0.13 ^{def}
BC11	110.66±5.50 ^k	38.66±2.08 ^{cde}	37.00±1.00 ^{cd}	27.66±0.57 ^b	33.97±0.73 ^{bc}
BC13	64.00±3.00 ⁱ	39.33±1.52 ^{cde}	47.66±1.15 ^{fg}	19.66±1.52 ^a	28.47±1.12 ^b
CG	54.33±2.51 ^{gh}	32.66±1.52 ^{bc}	33.66±0.57 ^{bc}	15.33±1.15 ^a	18.00±1.00 ^a

Values are presented as mean±standard deviation. Values with different superscript within the same column are significantly different (p<0.05).

Table 3. Antioxidant activity and IC₅₀ of combination herbal drink

Sample	Antioxidant activity (%)					IC ₅₀ (mg/mL)
	0.5	1	1.5	2	2.5	
BB	40.59±6.89 ^g	57.49±7.58 ^h	55.35±1.67 ^h	72.93±5.01 ^{ij}	83.29±2.42 ^j	0.679±0.093 ^a
BC31	21.44±1.73 ^{bcd}	29.77±4.18 ^{defg}	36.54±9.23 ^{fg}	54.09±5.80 ^h	66.05±2.83 ^{hi}	1.758±0.133 ^b
BC11	16.74±0.50 ^{abc}	32.83±2.08 ^{efg}	36.31±2.58 ^{fg}	40.97±0.56 ^g	61.12±1.89 ^{hi}	2.140±0.074 ^b
BC13	12.34±0.96 ^{ab}	22.59±2.18 ^{bcde}	29.05±2.02 ^{defg}	27.37±3.72 ^{cdef}	21.52±1.42 ^{bcde}	3.798±0.145 ^c
CG	10.62±2.30 ^{ab}	16.39±2.42 ^{abc}	15.78±1.04 ^{abc}	19.30±4.82 ^{abcd}	8.85±0.46 ^a	7.376±0.253 ^d

Values are presented as mean±standard deviation. Values with different superscript within the same column are significantly different (p<0.05).

to 66.05±2.83% (Table 3). The *B. balsamifera* L leaf extract produces antioxidant activity of 40.59±6.89 to 83.29±2.42%, while the *C. grandis* L leaf extract produces antioxidant activities of 8.85±0.46 to 19.30±4.82%. The combination of *B. balsamifera* L and *C. grandis* L in ratio 3:1 and *B. balsamifera* L and *C. grandis* L in ratio 1:1 shows an IC₅₀ value that is not significantly different from its ability to free radical scavenger. The combination of *B. balsamifera* L and *C. grandis* L in ratio 3:1 and *B. balsamifera* L and *C. grandis* L in ratio 1:1 has the ability to the highest free radical scavenger because it has a low IC₅₀ value. The *B. balsamifera* L leaf extract has an IC₅₀ value of 0,679±0,093 mg/ml, while the *C. grandis* L leaf extract has an IC₅₀ value of 7.376±0.25 mg/mL.

3.4 α -amylase activity

The combination of *B. balsamifera* L and *C. grandis* L drink resulted in the inhibitory ability of α -amylase 29.65±0.06 to 79.72±0.06% (Table 4). The *B. balsamifera* L amounted to 37.23±1.04 to 85.90±0.06%,

while the *C. grandis* leaf extract has an α -amylase activity of 12.10±0.41 to 23.28±0.11%. Tamilselvan *et al.*, (2011) the leaves of the extract extracted with methanol have an 81.13% inhibitory activity of α -amylase. The combination of *B. balsamifera* L and *C. grandis* L in ratio 3:1 drink at concentrations of 2 g produces the highest α -amylase activity, while the combination of *B. balsamifera* L and *C. grandis* L in ratio 1:3 at a concentration of 0.5 g results in the lowest α -amylase activity. The combination of *B. balsamifera* L and *C. grandis* L in ratio 3:1 drink produces an IC₅₀ of 1.109±0.067 mg/mL, while the *C. grandis* L leaf extract has an IC₅₀ of 7.517±0.265 mg/mL. Acarbose as a positive control has an IC₅₀ value of 4.35±0.05 mg/mL.

3.5 Evaluation of parameters across tests

There was significant (p<0.01) positive correlation between the total flavonoid content (R = 0.042), total antioxidant activity (R = 0.331) and α -amylase (R = 0.161) for combination drink. A negative correlation was observed between total phenolic content (R = -0.316).

Table 4. α -amylase inhibitory activity dan IC₅₀ of combination herbal drink

Sample	α -amylase inhibitory activity (%)					IC ₅₀ (mg/mL)
	0.5	1	1.5	2	2.5	
BB	51.17±0.25 ^h	52.87±0.61 ^h	76.45±0.06 ^j	77.52±0.35 ^j	88.33±0.52 ^k	0.526±0.010 ^a
BC31	45.80±0.68 ^{efg}	43.92±1.43 ^{def}	49.74±0.35 ^{gh}	62.14±3.96 ⁱ	79.72±0.06 ^j	1.109±0.067 ^b
BC11	42.08±1.73 ^{de}	42.93±1.34 ^{de}	44.51±0.06 ^{ef}	48.45±1.33 ^{fgh}	62.10±3.92 ⁱ	1.737±0.116 ^c
BC13	29.65±0.06 ^c	32.08±2.64 ^c	39.07±2.29 ^d	41.28±1.82 ^{de}	42.75±1.49 ^{de}	3.359±0.338 ^d
CG	12.10±0.41 ^a	19.24±0.16 ^b	20.08±0.11 ^b	22.33±0.35 ^b	23.28±0.11 ^b	7.517±0.265 ^e

Values are presented as mean±standard deviation. Values with different superscript within the same column are significantly different (p<0.05).

4. Discussion

4.1 Total flavonoid content

The addition of *B. balsamifera* L extracts significantly increases the TFC ($P < 0.05$). Plants that showed hypoglycemic effects are generally rich in alkaloids, steroids, saponins, tannins, flavonoids, cardiac glycosides (Sudha *et al.*, 2011), terpenes, and phenolic (Birari and Bhutani, 2007). *Blumea balsamifera* L extracted with ethanol and water (70:30%) are reported to have flavonoids, tannins, and polyphenols (Roy *et al.*, 2013), saponins, and terpenes (Pang *et al.*, 2014) which act as antidiabetic. In addition, ethanolic and water extract of *C. grandis* L contain high beta carotene. Ethanolic and water extract of *C. grandis* L was reported as a hypoglycemic agent for the treatment of diabetes (Tamilselvan *et al.*, 2011). Rodrigues *et al.* (2019) reported an increase in the total flavonoid content after the addition of other plant materials.

4.2 Total phenolic content

In this study, all treatments showed a significant increase ($p < 0.05$) in phenolic content. The addition of *B. balsamifera* L leaves improved the TPC in this combination drink. The phenolic bioactive content is relevant to health and associated with antioxidant and anti-hyperglycemic (Mishra *et al.*, 2019). Plant extracts that have high phenolic content can be used to prevent type 2 diabetes, because phenolic components are able to inhibit α -amylase and α -glucosidase (Damager *et al.*, 2004). Boiling extraction increase the total content of phenolic in the herbal drink. High temperatures will facilitate the release of bioactive components that are firmly bound to the material (Kusumawati *et al.*, 2018). Gonçalves Rodrigues *et al.* (2019) reported that an increase in temperature would result in an increase in total phenolic content. The heating process would lead to an increase in biological activity due to the changes in chemical compounds. In which, breakage of cellulose could increase the availability of bioactive compounds (Kim *et al.*, 2013).

4.3 Antioxidant activity

The addition of *B. balsamifera* L in this combination drink, resulting in an increase in antioxidant activity. Similar to Rodrigues *et al.* (2019), the combination of the lavender leaf (*Limonium algarvense* Erben) with green tea (*Camellia sinensis* L.) showed increased antioxidant activity in inhibiting free radicals. Studies by Shaw *et al.*, 2010 have shown that methanolic extract of *C. grandis* L leaf powder exhibits high antioxidant activity when compared to water extracts. In a similar manner, Roy *et al.* (2013) reported that *B. balsamifera* L powdered leaves extracted with 70% ethanol and 30%

water was able to increase the antioxidant activity of Glutathione (GSH) and catalase (CAT) in rats suffering from diabetes. Antioxidant activity in the combination of herbal drinks can cause various effects. Combining herbs can enhance the pharmacological properties as well as reduce the toxicity effect. Interactions that may occur as a result of the combination can be antagonistic, synergistic, or additive interactions (Rodrigues *et al.*, 2019). IC_{50} is the inhibitory concentration carried out by antioxidants in reducing 50% free radicals. The low value of IC_{50} shows high antioxidant power (Brand-Williams *et al.*, 1995). This suggests that the addition of *B. balsamifera* L leaf extract is able to improve the ability to free radicals scavenging in this combination drink.

4.5 α -amylase activity

The addition of *B. balsamifera* L leaf extract in combination drinks was able to improve the inhibitory ability of α -amylase. Extracts that have a value of IC_{50} less than 2 mg/mL are effective in inhibiting α -amylase (Podsędek *et al.*, 2014). Roy *et al.* (2013) reported that the *B. balsamifera* L leaves were extracted with 70% ethanol and 30% water significantly reduced the elevated glycosylated haemoglobin levels in STZ diabetic rats. Mohammed *et al.* (2016) report that the extract of the *C. grandis* L leaf in ethanol has the smallest IC_{50} value. This suggests that ethanol extracts have the best α -amylase inhibitory ability when compared to ethyl acetate and hexane. Inhibition of α -amylase may decrease blood glucose synthesis thereby decreasing the absorption of carbohydrates and obstructing glucose transport (Chhetri *et al.*, 2005). The ability to extract the *C. grandis* L leaf inhibits α -amylase and could suppress the risk of T2DM (Tamilselvan *et al.*, 2011). The combination of BC drinks showed its ability to inhibit α -amylase so that this combination of drinks can be used to prevent the risk of diabetes.

4.6 Evaluation of parameters across tests

There is a positive correlation between total antioxidant activity, total flavonoid content, and α -amylase in combination drinks. High flavonoid content showed high antioxidant activity as well, there is a link between flavonoids and antioxidant activity (Sy *et al.*, 2017). Some medicinal plants have an antidiabetic ability due to the presence of flavonoids (Zaidan *et al.*, 2019) by means of inhibiting α -amylase activities (Yilmazer-Musa *et al.*, 2012). We also observed that there is no correlation between total antioxidant activity and phenolic contents in combination drinks. Ivanova *et al.* (2005) report that not all phenolic compounds have radical scavenging activity. The extraction method of a combination drink by boiling may give different results

in the antioxidant activity. For extraction of active components required selective organic solvents.

5. Conclusion

In conclusion, the presence of TFC was revealed to contribute to the antioxidant activity and α -amylase inhibition activity in combination herbal drinks. The combination of *B. balsamifera* L and *C. grandis* L in ratio 3:1 was found to be the best formulation combination herbal drink with promising α -amylase inhibition activity. The antioxidant and α -amylase inhibitory activities of BC are related to their flavonoid compound.

Conflict of interest

There is no conflict of interest.

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