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Antioxidant properties of extract combination of *Coccinia grandis* and *Blumea balsamifera*: An *in vitro* synergistic effect

I Made Wisnu Adhi Putra[1,2], Nanang Fakhrudin[3*], I Gusti Ayu Wita Kusumawati[4], Arief Nurrochmad[5], Subagus Wahyuono[3]

[1]Doctorate Program of Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
[2]Department of Biology, University of Dhyana Pura, Kuta Utara, Badung, Bali 80361, Indonesia
[3]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
[4]Department of Nutrition Science, University of Dhyana Pura, Kuta Utara, Badung, Bali 80361, Indonesia
[5]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

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ABSTRACT

Introduction: As single extracts, *Coccinia grandis* and *Blumea balsamifera* have been known to have potent antioxidant activities. However, the synergistic antioxidant effect of the combination of these plant extracts was unknown. In this study, the combination of *C. grandis* and *B. balsamifera* extracts was investigated for its antioxidant and synergistic properties.
Methods: Separately, *C. grandis* and *B. balsamifera* leaves were extracted with ethanol. After evaporation, the thick extracts were assayed for their total phenolic content (TPC) and total flavonoid content (TFC). The antioxidant properties of single and combined extracts were measured using the molybdenum(VI) reducing power, ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods. The possible synergism effect was evaluated using the checkerboard method and the combination index values were also calculated.
Results: The TPC and TFC of the *B. balsamifera* extracts were much greater than that of *C. grandis* extract. In the molybdenum(VI) reducing power and FRAP assay, the reducing power of the extract combination increased as *B. balsamifera* extract concentration increased ($P<0.05$). In the ABTS+ and DPPH radical scavenging assays, *B. balsamifera* extract demonstrated a higher antioxidant activity than *C. grandis* extract ($P<0.05$). When combined, increasing the concentration of *B. balsamifera* caused an increase in the radical scavenging activity ($P<0.05$). Synergism was observed in the combination of the extracts with low concentration ratios.
Conclusion: In this study, we showed that the combination of *C. grandis* and *B. balsamifera* leaf extracts possessed synergistic antioxidant properties.

Implication for health policy/practice/research/medical education:
The combination of *Coccinia grandis* and *Blumea balsamifera* showed a synergistic effect in scavenging free radicals. This research can be continued to the *in vivo* assay to obtain more evidences for the efficacy of this combined extract in the biological environment. The results can be used as an alternative herbal medicine to overcome health problems related to oxidative stress.
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Introduction

Oxidative stress refers to a state in which there is an imbalance between the production of reactive oxygen species (ROS) in cells and tissues and the ability of biological systems to degrade these reactive compounds (1). Excessive production and accumulation of ROS in the body have been associated with various diseases, including cancer, cardiovascular disease, neurological disease, respiratory disease, rheumatoid arthritis, kidney disease, and sexual maturation disorders (2). Naturally, the body produces endogenous antioxidant molecules, such as glutathione peroxidase (GPx), catalase (CAT),

***Corresponding author**: Nanang Fakhrudin,
Email: nanangf@ugm.ac.id

and superoxide dismutase (SOD), to fight ROS. However, the amount of those molecules is not sufficient when the level of ROS exceeds the internal antioxidant capacity of the human body (3). This imbalance can trigger oxidative stress. Therefore, we need exogenous antioxidants that enhance body's antioxidant capacity.

Natural antioxidants derived from plants contain bioactive compounds that can stabilize free radicals. Polyphenols (flavonoids, tannins) are secondary metabolites produced by plants, proven to have strong antioxidant properties (4). The antioxidant properties are mainly due to their oxidation-reduction properties enabling phenolic compounds to act as reducing agents, proton donors, free radical scavengers, and peroxide decomposers (5). *Coccinia grandis* (L.) Voigt is a medicinal plant that has been used traditionally for generations in India and Sri Lanka to treat various ailments, including jaundice and diabetes. This plant has been reported to have antioxidant properties in several *in vitro* assays (6-9). Although *C. grandis* extract has demonstrated antioxidant properties, its efficacy needs to be increased without drastically increase the dose given to avoid the possible side effects. One of the approaches to boost the antioxidant activity is by combining the *C. grandis* extract with other plants. When extracts with antioxidant properties are combined, a synergistic effect might occur and produce stronger antioxidant effect than the sum produced by the single extract (additive) (10). For example, the combination of *Osmanthus fragrans* flower extract with 4 types of tea (Longjing tea, black tea, Tieguanyin tea, and Pu'er Tea) showed a synergistic effect in scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals (11).

Blumea balsamifera is one of the plants containing high amount of phenolics and flavonoids (12) having the potential to be combined with *C. grandis*. For thousands of years, this plant has been used in traditional medicine in Southeast Asian countries, including Thailand, the Philippines, Vietnam, and Malaysia (13). The antioxidant activity of *B. balsamifera* extract has been reported in the literature (14,15). All petroleum ether, chloroform and methanol extracts of *B. balsamifera* leaves were able to inhibit xanthine oxidase activity and to scavenge superoxide radicals (16). Additionally, the ethanol extract of *B. balsamifera* leaf was reported to have the ability to scavenge DPPH free radicals and the ability to inhibit tyrosinase (17). *B. balsamifera* leaves extracted using two different extraction methods (infusion and decoction) showed high antioxidant capacity values (by DPPH and FRAP [ferric reducing antioxidant power] methods) (18).

In this study, the antioxidant activities of *C. grandis* and *B. balsamifera* leaves extracts were studied using the molybdenum(VI) reducing power, FRAP, DPPH free radical scavenging, and ABTS+ radical scavenging methods. The synergistic effect of the combination of those extracts was calculated using the combination index (CI) equation. This research provides scientific evidence regarding the utilization of herbal medicine combinations to increase the antioxidant effect.

Materials and Methods

Chemicals

Ethanol, methanol, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, aluminum chloride, potassium acetate, quercetin, hydrochloric acid, and toluene were purchased from Merck KGaA (Darmstadt, Germany). Ammonium molybdate, ferric chloride, ascorbic acid, Trolox, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), DPPH, potassium persulfate, and 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were purchased from Sigma-Aldrich PTE LTD (Singapore).

Plant material

C. grandis leaves were harvested on June 6-7, 2020, in Tabanan Regency, Bali, Indonesia. *B. balsamifera* leaves were harvested in Bondowoso Regency, East Java, Indonesia, on May 20, 2020. Both plants were identified at Bali Botanic Garden, Indonesian Institute of Science, with identification and herbal number B-192/IPH.7/AP/VII/2020.

Preparation of *C. grandis* and *B. balsamifera* extracts

Separately, the fresh leaves of *C. grandis* and *B. balsamifera* were washed with running water and drained at room temperature. The leaves were then oven-dried for 3 days at 45°C and crushed into a fine powder. The dried powder of the plant materials was extracted using the maceration method according to a previous study with a few modifications (19). One kilogram of the dried plant powder was macerated with ten liters of ethanol (70%) for 24 hours, stirring every 3 hours. This maceration process was repeated 3 times. The macerates were then concentrated using a rotary evaporator and then putting in a desiccator until dryness.

Characterization of the extracts

The extraction yield, water content, total phenolic content (TPC), and total flavonoid content (TFC) were determined based on the protocols described in Indonesian Herbal Pharmacopoeia (20).

Molybdenum(VI) reducing power assay

Mo(VI) reducing power assay was carried out following the method presented by Bhatti et al (21) with a few modifications. In a test tube, the extract solution was mixed with 2000 µL of reagent and distilled water to produce a final concentration of 100 µg/mL. The mixture was vortexed and incubated at 95°C for 90 minutes. The absorbance of the sample was measured using a UV-Vis spectrophotometer (Genesys 10S) at 693 nm. The absorbance value obtained was entered into the linear regression equation of the ascorbic acid solution ($y = 0.0414 + 0.0296$; $R^2 = 0.998$). The antioxidant power

was expressed in mg AAE/g extract and was measured using the following equation:

$$AP = \frac{C \times V \times DF \times 10^{-3}}{M} \quad (1)$$

Where AP: antioxidant power (mg AAE/g extract), C: concentration of extract (µg AAE/ml), V: volume of extract solution (ml), DF: dilution factor, M: mass of extract (g).

Ferric reducing antioxidant power (FRAP) assay

This assay was carried out following the method presented by Nurrochmad et al (22) with modifications. In a cuvette, 200 µL of stock extract solution (1000 µg/mL) was mixed with 1300 µL of methanol and 500 µL of FRAP reagent to produce a final extract concentration of 100 µg/mL. The mixture was then incubated for 15 minutes at 37°C. The sample absorbance value obtained (at wavelength of 594 nm) was entered into the linear regression equation of the ascorbic acid solution ($y = 0.2095x + 0.2353$; $R^2 = 0.9991$). The antioxidant power was expressed in mg AAE/g extract and was measured using the equation 1.

ABTS+ radical scavenging activity assay

The ABTS+ free radical scavenging activity assay was carried out following the method presented by Lee et al (23) with modifications. One ml of *C. grandis* extract solution (33-200 µg/mL) was mixed with 2 mL of ABTS+ reagent and incubated in a dark room (protected from light) for 6 minutes. The absorbance was measured spectrophotometrically at 516 nm. The same procedure was carried out on *B. balsamifera* extract (7-67 µg/mL). A solution mixture containing methanol instead of extract was treated as a control. Percentage of ABTS free radical scavenging was calculated using the following equation:

$$\%\text{Radical scavenging} = \frac{Ac - As}{Ac} \times 100\% \quad (2)$$

Where Ac: Absorbance of control and As: Absorbance of sample. The inhibitory concentration (IC_{50}) value of all samples was calculated based on the linear regression equation $y = ax + b$ which was generated from the plot of sample concentration vs %radical scavenging.

DPPH radical scavenging activity assay

The DPPH radical scavenging activity assay was carried out following the method presented by Roy et al (24) with modifications. A total of 2 ml of *C. grandis* extract solution (100-500 µg/mL) was mixed with 2 mL of DPPH solution (70 µg/mL) and incubated in a dark room (protected from light) for 30 minutes. The absorbance was measured at 516 nm. The same procedure was carried out on *B. balsamifera* extract (10-50 µg/mL). A solution mixture containing methanol instead of extract was treated as a control. Percentage of DPPH free radical scavenging activity was calculated using the equation 2. The inhibitory concentration (IC_{50}) value of all samples was calculated based on the linear regression equation $y = ax + b$ which was generated from the plot of sample concentration vs %radical scavenging.

Determination of antioxidant synergism

Antioxidant synergism was calculated based on the combination of IC_{50} values of each single extract. There were 3 solution concentration series made, namely: ¼IC_{50}, ½IC_{50}, and IC_{50}. These concentration series were then combined based on checkerboard method so as to produce a total of 9 solution combinations. The absorbance of the solution combination was then measured to obtain the percentage of radical scavenging activity. The calculation of CI is used to determine whether a combination has synergistic effect or not. The equation for determining CI is expressed as follows (25,26):

$$CI = \frac{D_{1(x)}}{E_{1(x)}} + \frac{D_{2(x)}}{E_{2(x)}} \quad (3)$$

Where $D_{1(x)}$ and $D_{2(x)}$ are the concentration of each combined extract that produces the x effect, $E_{1(x)}$ and $E_{2(x)}$ are the concentration of each single extract that produces the x effect. A CI value < 1 indicates a synergistic interaction, CI = 1 indicates an additive interaction, and a CI > 1 indicates an antagonistic interaction (27).

Statistical analysis

The data were presented as the mean ± standard deviation (SD) and the experiment was carried out in at least in a triplicate. Statistical analysis was done using the normality test followed by the analysis of variance (ANOVA) test, and Tukey post hoc test. This statistical analysis was performed in SPSS v. 24 software (IBM).

Results

Characteristics of the extracts

The characteristics of *C. grandis* and *B. balsamifera* extracts are shown in Table 1. The extraction yield of *C. grandis* leaves (21.12%) was much higher than that of *B. balsamifera* leaves (9.10%). The value of TPC and TFC of *B. balsamifera are* 4.39 ± 0.09 % GAE and 2.54 ± 0.04 % QE, respectively. These values are significantly higher than that of *C. grandis* extract ($P < 0.05$) which has TPC and TFC values of 1.85 ± 0.03 % GAE and 0.83 ± 0.02 % QE, respectively. The water content of *C. grandis* (8.66 ± 0.58 % mL/g) and *B. balsamifera* (8.33 ± 0.57 % mL/g) shows no significant difference ($P > 0.05$).

Molybdenum(VI) reducing power assay

The results of Mo(VI) reducing power assay of the leaves extract of *C. grandis*, *B. balsamifera*, and their combinations are shown in Figure 1. The antioxidant power of all extracts shows a significant difference ($P < 0.05$). *B. balsamifera* leaves extract had an antioxidant

Table 1. Characteristics of the extracts

Samples	Yield (%)	Water Content (%mL/g)*	Total Phenolic Content (%GAE)*	Total Flavonoid Content (%QE)*
C. grandis	21.12	8.66±0.58	1.85±0.03	0.83±0.02
B. balsamifera	9.10	8.33±0.57	4.39±0.09	2.54±0.04

GAE = gallic acid equivalent; QE = quercetin equivalent. Values were represented in the mean ± standard deviation (n=3)
*Significance was determined using independent *t* test ($P < 0.05$).

power (11.324 ± 0.032 mg AAE/g extract), which was much higher than that of *C. grandis* extract (4.791 ± 0.056 mg AAE/g extract). When *C. grandis* extract was combined with *B. balsamifera* extract in a ratio of 1:3, 1:1, and 3:1, the antioxidant power of the combined extracts became 8.128 ± 0.149, 6.357 ± 0.032, and 5.514 ± 0.118 mg AAE/g extract, respectively. These results indicated that the combined extract had higher antioxidant power than the single extract of *C. grandis*. However, when compared to *B. balsamifera* extract, the combined extract had a lower antioxidant power.

FRAP assay

The results of the FRAP assay of all extracts (*C. grandis*, *B. balsamifera*, and their combinations) are shown in Figure 2. There was a significant difference in the antioxidant power between the single extracts and their combinations ($P < 0.05$). Similar to the Mo(VI) reducing power assay, the FRAP value of *B. balsamifera* extract (1.637 ± 0.080 mg AAE/g extract) was higher than that of *C. grandis* extract (0.764 ± 0.061 mg AAE/g extract). The combination of *C. grandis* and *B. balsamifera* extracts with ratios of 1:3, 1:1, and 3:1 gave the antioxidant power of 1.323 ± 0.027, 1.202 ± 0.015, and 0.933 ± 0.028 mg AAE/g extract, respectively.

ABTS+ radical scavenging activity assay

The results of ABTS+ radical scavenging activity assay are shown in Table 2. There was a significant difference in radical scavenging activity between the extract of *C. grandis* and *B. balsamifera* ($P < 0.05$). *B. balsamifera* extract had a much stronger activity (IC_{50} = 32.970 ± 0.222 µg/mL) in scavenging ABTS+ radical than *C. grandis* extract (IC_{50} = 67.960 ± 1.324 µg/mL). This can be seen from the IC_{50} value of *B. balsamifera* extract, which is lower than that of *C. grandis* extract. When compared with Trolox as a positive control, both extracts had lower scavenging activity.

DPPH radical scavenging activity assay

Based on the results (Table 2), *B. balsamifera* extract had a much stronger DPPH radical scavenging activity (IC_{50} = 32.022 ± 0.086 µg/mL) than *C. grandis* extract (IC_{50} = 279.964 ± 1.791 µg/mL). However, the activity of *B. balsamifera* extract in scavenging DPPH radicals was still inferior to the positive control, namely Trolox.

Antioxidant synergism

Figure 3 shows the ABTS+ radical scavenging activity of the combination of *C. grandis* and *B. balsamifera* extracts. The combination of *C. grandis* and *B. balsamifera*

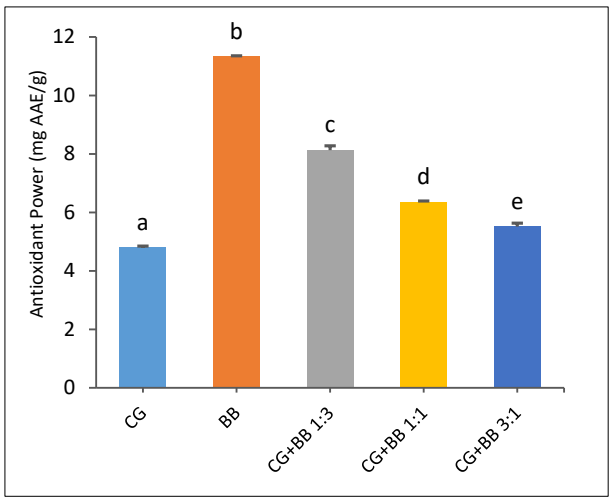

Figure 1. Molybdenum(VI) reducing power activity of the extracts of *C. grandis* (CG), *B. balsamifera* (BB), and their combinations with various ratios. Error bars represent the standard deviation (n=3). AAE = ascorbic acid equivalent. Different letters (a-e) indicate significant differences based on ANOVA followed by Tukey post-hoc test ($P < 0.05$).

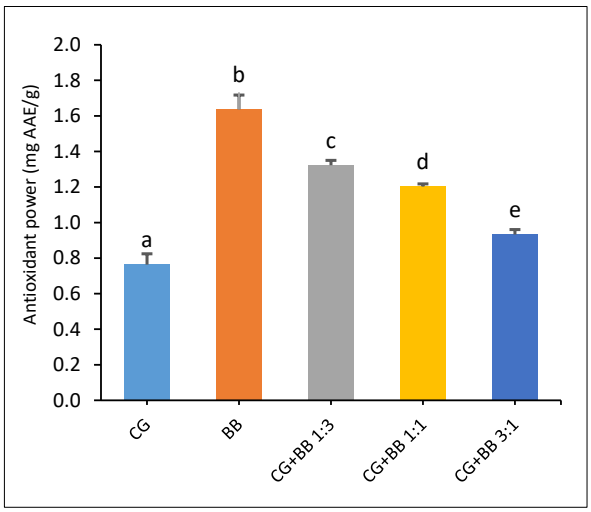

Figure 2. Ferric reducing antioxidant power (FRAP) of the extracts of *C. grandis* (CG), *B. balsamifera* (BB), and their combinations with various ratios. Error bars represent the standard deviation (n=3). AAE = ascorbic acid equivalent. Different letters (a-e) indicate significant differences based on ANOVA followed by Tukey post-hoc test ($P < 0.05$).

Table 2. $ABTS^+$ and DPPH radicals scavenging activities of the extracts

Samples	$ABTS^+$ Scavenging Activity, IC_{50} (µg/mL)	DPPH Scavenging Activity, IC_{50} (µg/mL)
C. grandis	67.960±1.324[a]	279.964±1.791[a]
B. balsamifera	32.970±0.222[b]	32.022±0.086[b]
Trolox	2.431±0.014[c]	5.979±0.036[c]

ABTS = 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; DPPH = 2,2-diphenyl-1-picrylhydrazyl; IC_{50}= Half maximal inhibitory concentration. Values are represented as mean ± standard deviation (n=3). Different letters (a-c) indicate significant difference based on ANOVA followed by Tukey test ($P < 0.05$).

extracts produced a significant difference in the % radical scavenging values ($P<0.05$). The increase in the concentration ratio of *B. balsamifera* in the combination led to an increase in the % radical scavenging value. The highest % radical scavenging value (68.329 ± 0.810%) was obtained by combination 9. However, synergism was shown by combinations 1 and 4 with CI values of 0.86 and 0.97, respectively (Table 3).

Similar to the ABTS+ radical scavenging assay, the combination of *C. grandis* and *B. balsamifera* extracts also showed a significant difference in the % radical scavenging values against DPPH radicals ($P<0.05$) (Figure 4). Antioxidant synergism was achieved by a combination of 1-7 with CI values of 0.52, 0.72, 0.81, 0.69, 0.86, 0.90, and 0.91, respectively. Combinations of 8 and 9 showed antagonistic interactions (Table 3).

Discussion

In this study, the reducing power of *C. grandis* and *B. balsamifera* was evaluated using Mo(VI) reducing power and FRAP assays. The results of Mo(VI) reducing power assay (Figure 1) showed that the antioxidant power of *C. grandis* was much greater than that of *B. balsamifera*. This was caused by the higher total phenolic and flavonoid contents of *B. balsamifera* extract compared with *C. grandis* extract (Table 1). Phenolics and flavonoids have powerful antioxidant activities (28). The FRAP assay also showed the same results where the extract of *B. balsamifera* had a much higher antioxidant power than the extract of *C. grandis* (Figure 2). The antioxidant power of the combination of *C. grandis* and *B. balsamifera* extracts increased with the increasing concentration of *B. balsamifera* extract. This effect was observed in the results of the Mo(VI) reducing power and the FRAP assays. This seems reasonable considering that the phenolic and flavonoid contents in the extract of *B. balsamifera* were higher than that of *C. grandis*. The reducing power of the combination of several extracts was reported by Xu et al. (29). They showed that the combination of *Astragalus membranaceus* and *Paeonia lactiflora* prepared by bioactivity-guided fractionation had a powerful reducing power. Crespo et al (30) also revealed that the combination of *Apium graveolens* and *Coriandrum sativum* had higher reducing power than single extracts. Furthermore, a higher reducing power was also obtained by the addition of *Thymus vulgaris* to the mixtures.

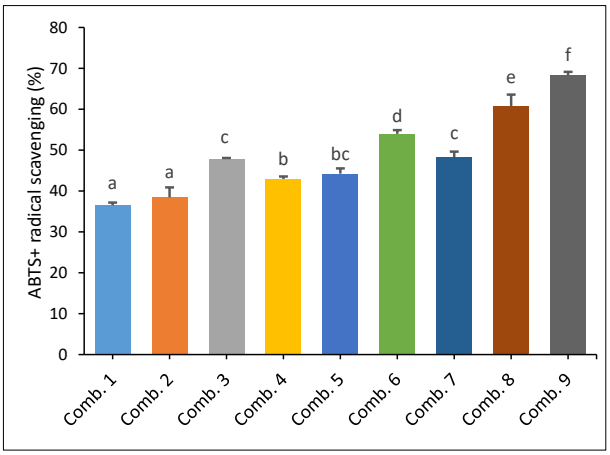

Figure 3. $ABTS^+$ radical scavenging activity of the combination of the extracts. The combination of *C. grandis* and *B. balsamifera* extracts with ratios of ¼IC_{50}:¼IC_{50} (Comb. 1), ¼IC_{50}:½IC_{50} (Comb. 2), ¼IC_{50}:IC_{50} (Comb. 3), ½IC_{50}:¼IC_{50} (Comb. 4), ½IC_5:½IC_{50} (Comb. 5), ½IC_5:IC_{50} (Comb. 6), IC_{50}:¼IC_{50} (Comb. 7), IC_{50}:IC_{50} (Comb. 8), IC_{50}:IC_{50} (Comb. 9). Comb.= combination; ABTS = 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid. Error bars represent the standard deviation (n=3). Different letters (a, b, bc, c, d-f) indicate significant difference (ANOVA followed by Tukey test ($P < 0.05$).

The results of the ABTS+ and DPPH radical scavenging assays (Table 2) showed a relationship between the phytochemical content of the extract and the antioxidant activity. *B. balsamifera* extract was a very strong antioxidant in all assays (IC_{50}<50 g/mL), meanwhile *C. grandis* was a strong (IC_{50}: 50-100 µg/mL) and weak antioxidant (IC_{50}>150 µg/mL) in ABTS+ and DPPH radical scavenging assays, respectively. Although the extracts of *C. grandis* and *B. balsamifera* have the ability to scavenge free radicals, this does not necessarily apply to real biological systems. Further *in vivo* experiment is required to confirm this efficacy. However, the extracts at least showed antioxidant properties through both single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms. In the HAT method, antioxidant activity is measured based on the capture of free radicals by antioxidants via proton donors, while the SET method measures antioxidant activity through electron transfer from antioxidants to free radicals (31).

The combination of *C. grandis* and *B. balsamifera* extracts showed concentration ratios dependent manner

Table 3. The combination index (CI) values of the ABTS+ and DPPH radical scavenging activities of the combination of *C. grandis* and *B. balsamifera* extracts

Ratio		Sample Codes	ABTS+ radical scavenging		DPPH radical scavenging	
C. grandis	*B. balsamifera*		CI	Remarks	CI	Remarks
1/4 IC_{50}	1/4 IC_{50}	Comb. 1	0.86	Synergism	0.52	Synergism
1/4 IC_{50}	1/2 IC_{50}	Comb. 2	1.13	Antagonism	0.72	Synergism
1/4 IC_{50}	IC_{50}	Comb. 3	1.32	Antagonism	0.81	Synergism
1/2 IC_{50}	1/4 IC_{50}	Comb. 4	0.97	Synergism	0.69	Synergism
1/2 IC_{50}	1/2 IC_{50}	Comb. 5	1.21	Antagonism	0.86	Synergism
1/2 IC_{50}	IC_{50}	Comb. 6	1.35	Antagonism	0.90	Synergism
IC_{50}	1/4 IC_{50}	Comb. 7	1.33	Antagonism	0.91	Synergism
IC_{50}	1/2 IC_{50}	Comb. 8	1.12	Antagonism	1.01	Antagonism
IC_{50}	IC_{50}	Comb. 9	1.29	Antagonism	1.17	Antagonism

ABTS = 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; DPPH = 2,2-diphenyl-1-picrylhydrazyl; IC_{50} = the half maximal inhibitory concentration. Comb: Combination.

(Figure 3 and Figure 4). In both free radical scavenging assays (ABTS+ and DPPH radical scavenging assays), the increase of *B. balsamifera* concentration ratios resulted in the increase of radical scavenging activity. Interestingly, the synergistic effects were achieved in combining the extracts at a relatively low concentration (Table 3). The interaction between antioxidants in the combination of the extracts is strongly influenced by the concentration and the ratio of bioactive components of the extracts (32,33). In combination with a low IC_{50} ratio, synergism was observed. This showed that the total antioxidant effect in the ABTS+ and DPPH radicals scavenging assays was stronger than the sum of the single antioxidant effects. The mechanism occurring synergism at low concentrations is not yet understood. However, it may be due to antioxidant regeneration (32). In low concentration, the amount of *C. grandis* extracts (weaker antioxidant) was sufficient to regenerate *B. balsamifera* extract (stronger antioxidant). However, antagonistic effects occurred in combinations with higher IC_{50} ratios. This might be due to the high concentration of phenolic and flavonoid compounds in the combined extract, which caused the antioxidant regeneration effect to decrease and caused them to compete with each other. The higher the concentration of the single extract used in the combination, the more antagonistic interactions occur (34). This antagonistic interaction was fully observed by Pratoomsoot et al (35). They revealed that the combination of *Acanthus ebracteatus* + *Clerodendrum inerme, A. ebracteatus* + *C. grandis,* and *C. inerme* + *C. grandis* extracts exhibited antagonistic antioxidant effects.

Conclusion

The combination of *C. grandis* and *B. balsamifera* extracts showed promising antioxidant activity. The reducing power of the combined extract increased with the increasing concentration of *B. balsamifera* extract. This seems reasonable considering the total phenolic

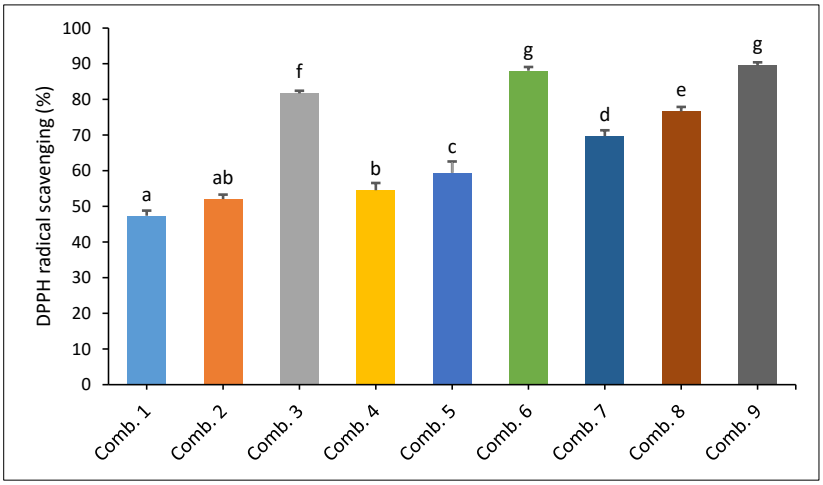

Figure 4. DPPH radical scavenging activity of the extracts. The combination of *C. grandis* and *B. balsamifera* extracts with ratios of ¼IC_{50}:¼IC_{50} (Comb. 1), ¼IC_{50}:½IC_{50} (Comb. 2), ¼IC_{50}:IC_{50} (Comb. 3), ½IC_{50}:¼IC_{50} (Comb. 4), ½IC_{5}:½IC_{50} (Comb. 5), ½IC_{5}:IC_{50} (Comb. 6), IC_{50}:¼IC_{50} (Comb. 7), IC_{50}:IC_{50} (Comb. 8), IC_{50}:IC_{50} (Comb. 9). Comb.= combination; DPPH = 2,2-diphenyl-1-picrylhydrazyl. Error bars represent the standard deviation (n=3). Different letters (a, b, ab, c, c-g) indicate significant difference (ANOVA followed by Tukey test, $P < 0.05$).

and flavonoid contents of *B. balsamifera* extract was much higher than that of *C. grandis* extract. Antioxidant synergistic effects were observed in low concentration ratios of the combination, which might be due to antioxidant regeneration.

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Authors' contributions

IMWAP and IGAWK performed the work, analyzed the results and drafted the manuscript. NF, AN, and SW conducted critical reviews to the manuscript prior to submit to the journal website.

Conflict of interests

The authors have no conflicts of interest regarding this investigation.

Ethical considerations

All authors have inspected the ethical issues of plagiarism, misconduct, data fabrication, falsification, double publication or redundancy related to the manuscript.

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