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Pachyrhizus erosus, tukak lambung, histopatologi lambung antioksidan, daun lengkeng, Dimocarpus longan, lotion asam 4-benzoiloksisinamat, microwave, daya iradiasi gelombang mikro, asam p-kumarat demam berdarah, kristaloid, koloid dismenorea, kunyit, asam jawa, kedelai drug related problems, RSUD Dr M Yunus Bengkulu, stroke, SF-36 fenolik total, antioksidan, sediaan Myrmecodia pendens fondaparinux, lama pemberian, activated partial thromboplastin time, sindroma koroner akut levofloxacin, MDR, Pseudomonas aeruginosa, Staphylococcus aureus lipstik, timbal (Pb), spektrofotometer serapan atom madu, produk madu, Surabaya metformin HCl, alginate, microspheres, amount of drug, aerosolization metronidazol, kitosan, natrium karboksimetilselulosa, hidrogel, desain faktorial mikrosfer, ekstrusi, natrium alginat, gelatin, probiotik Lactobacillus acidophilus obesitas, overweight, healthy weight management, apoteker perilaku, jilbab, kesehatan rambut perokok, motivasi, transtheoretical model (TTM), berhenti merokok pola terapi, penyakit ginjal kronis, kardioserebrovaskular solid lipid nanoparticles, coenzyme Q10, kosurfaktan, ukuran partikel, efisiensi penjebakan teh hijau, kafein, HPLC, penyeduhan vitamin B kombinasi, diabetes mellitus, neuropati diabetikum, TSS, SF-8

Effect of Guava Powder Addition on Epigallocatechin Gallate (EGCG) Content of Green Tea and Its Antioxidant Activity

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Abstract

***Background**: Tea (Camelia sinensis) contains polyphenols including epigallocatechin gallate (EGCG) which is acknowledged to have strong antioxidant properties. However, its stability is strongly influenced by environment. In a neutral and alkaline environment, EGCG could undergo degradation and lose its antioxidant property. There are some researches about the effect of combination of green tea and other plants to their antioxidant capacity. **Objective**: The research aimed to investigate the effect of guava addition to EGCG content of green tea and their antioxidant activity. **Methods**: The concentration of EGCG was determined by chromatographic analysis using TLC scanner, meanwhile the antioxidant activity was evaluated by its ability in scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) using UV-Vis Spectrophotometer. **Results**: Among all the samples, formula 4 (2 parts of green tea and 3 parts of guava) gave the highest EGCG content (39.03 ± 3.65 mg/g). This was 37.5% higher than the control sample (28.39 ± 2.45 mg/g). Formula 4 also had the best antioxidant activity with IC_{50} of 1917.32±1.75 ppm, 19% lower than control sample (2356.46 ± 3.16 ppm). **Conclusion**: The addition of guava powder significantly increased the amount of EGCG in green tea extracts and their antioxidant activity.*

***Keywords**: green tea, Camellia sinensis, guava, epigallocatechin gallate (EGCG), antioxidant activity*

INTRODUCTION

In recent years, tea (*Camellia sinensis*) has developed into the center of attention because of its health benefits, primarily as an antioxidant and anti-carcinogenic agents. Flavonoids are commonly believed to play important role in these benefits (Wang *et al.*, 2003). A glass of tea can contain about 300 - 400 mg of total polyphenols. Polyphenols have great antioxidant capacity and can shield body cells from the harmful side effects of reactive oxygen species. Dry tea leaves contain about 42% polyphenols in the form of catechins. The most common types of catechin inside the tea are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC). EGCG contributes to 13% of the total polyphenols in tea (Ramalakshmi & Rao, 2011).

One of the obstacles encountered during the application of tea is the stability of catechins. The stability of catechins including EGCG is strongly influenced by the environment, such as temperature, air, and pH during storage (Zeng *et al*., 2018). Tea catechins tend to be less stable at temperatures above 50°C, even during the heating time at 98°C; catechins can be degraded up to 20% (Chen *et al*., 2001). Catechin degradation will result in decreased antioxidant activity (Dhaouadi *et al*., 2016). Some solutions that have been applied to maintain the stability of tea catechin were keeping the tea inside refrigerator at 4°C, setting the pH of the solution around 4, and the addition of ascorbic acid (Chen *et al*., 1998). This was in concordance with the work by Fangueiro *et al.* (2014) which suggested that the existence of ascorbic acid provided great protection to EGCG against degradation.

The utilization of fruit has been known to reduce the degradation of catechins. Dhaouadi *et al.* (2016) reported that adding pomegranate to green tea significantly reduced the degradation of polyphenol content from 92% to 36% in storage for 15 days at 4°C. The use of lemon juice in tea drinks was also

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known to slow down the degradation of catechins (Bazinet *et al.,* 2010).

There are some researches about the effect of combination of green tea and other plants to their antioxidant capacity (Costa *et al*., 2012; Nedamani *et al.,* 2015*)*, however, the combination between green tea and pink guava has still not been evaluated. Guava was known to have a high level of ascorbic acid, flavonoids (Yan *et al.,* 2006), and phenolic content (Musa *et al.*, 2015). Therefore in this work, we evaluated the effects of guava addition on EGCG content were evaluated in green tea and their antioxidant activity. The concentration of EGCG was determined by chromatographic analysis using TLC scanner, meanwhile, the antioxidant activity was tested by sample's ability in capturing 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals using UV-Vis Spectrophotometer.

MATERIALS AND METHODS

Materials

(-)-Epigallocatechin gallate (EGCG) standard was purchased from Sigma (St. Louis, MO); 2,2-diphenyl-1-picrylhydrazyl (DPPH) was acquired from Sigma (Steinheim, Germany); methanol, chloroform, formic acid, and TLC plates (20 x 20 cm) pre-coated with silica gel 60 F254 were obtained from Merck (Darmstadt, Germany), ethyl acetate was obtained from Anhui Full Time (Anhui, China); acetone and distilled water were purchased from Smart Lab (Tangerang, Indonesia). All chemicals and reagents were analytical grades.

A commercial green tea (GT) product was purchased from local shop in Surabaya, Indonesia. Each sachet of the product contained 2 g of green tea powder. As for the fruit, pink guavas (GV) were collected from local market in Surabaya. The pink guavas were produced from plantation in Blitar, Indonesia. The ripe pink guavas which had good appearance were chosen for the study. The guavas were rinsed and peeled to remove the dirt. Clean guavas were then sliced into thin pieces and dried in fruit dehydrator for about 24 hours. Dried slices of guava were turned into powder using dry miller (Miyako Model BL-101 PL).

Instruments

Chromatographic analysis was performed using CAMAG TLC Scanner 4 (Muttenz, Switzerland) and the data was collected using winCATS software. Spectrophotometric analysis was carried out using Cary 60 UV-Vis Spectrophotometer from Agilent (California, US) with cuvettes of 1 cm length. XPE 26 Micro-analytical Balance from Mettler Toledo (Greinfensee, Switzerland) and STARTER 3000 bench pH meter were also used in this work.

Methods

Preparation of extracts

All extracts tested in this research were made freshly. Green tea powder (2 g) was put into 200 mL volumetric flask and added with 80°C distilled water until the mark. The solution was homogenized using magnetic stirrer for 10 minutes. Green tea extracts then were divided evenly for 5 variations as following: (1) green tea only (GTGV 2:0), (2) green tea added with 1 part of guava powder (GTGV 2:1), (3) green tea added with 2 parts of guava powder (GTGV 2:2), (3) green tea added with 3 parts of guava powder (GTGV 2:3), (4) green tea added with 4 parts of guava powder (GTGV 2:4). Every green tea extracts were then stirred again for another 5 minutes. All the extracts were then filtered to remove the undissolved particles. The extracts were kept in glass bottle at refrigerator if not in use.

Determination of EGCG content in extract

The extraction of EGCG in all formulas were performed using the method used by Kurniadi *et al.* (2007) with a minor adjustment. Initially, 2 mL of filtered samples were partitioned with 2 mL of chloroform. The chloroform layer was then removed. The water phased was collected and as a second partition, 2 mL of ethyl acetate was used. This step was repeated twice. The ethyl acetate layer was collected and evaporated inside acid chamber at room temperature. The extract obtained later was reconstituted with 2 mL of methanol. The quantification of EGCG was done using thin layer chromatography method which was previously used by Vasisht *et al.* (2003) with slight alteration. Each extracts (2 mL), and series of EGCG standards, were applied on pre-activated silica TLC plate. All quantitative analysis were made in three replicates. The plate was run in a chamber which was filled with chloroform, acetone, and formic acid with ratio of 5:4:1 as mobile phase. The plate was then dried inside acid chamber and scanned using Camag TLC scanner. The instrument setting as follows: slit dimensions 4.00 x 0.30 mm, scanning speed 20 mm/s, data resolution 100 µm/step, and wavelength 278 nm. The area under curve (AUC) of the EGCG peak was measured and the concentration was determined from the standard plots.

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Determination of antioxidant activity

The DPPH scavenging activities were carried out using the method developed by Dhaouadi *et al.* (2016) with slight modifications. First, the extracts were diluted with distilled water to make series of concentrations. The same amount of diluted extracts (40 µL) were then mixed with 1960 µL DPPH solutions (8.87 x 10^{-5} mol L^{-1}). The solutions were kept in dark for 60 minutes. The absorbance of blank (DPPH solution only) and every GTGV solutions were measured at 515 nm. The antioxidant activity of extracts was expressed as % inhibition of radical DPPH using equations as following $[(A_0-A_t)/A_0] \times 100$, where A_0 was the initial absorbance of DPPH solution only and A_t was the absorbance of GTGV solutions after 60 minutes incubation.

Statistical analysis

All test were done in three replicates. Values were presented as mean ± SD (n = 3). Statistical analysis was performed using SPSS software (Version 24, SPSS Inc.). One-way analysis of variance (ANOVA) was used to compare the means of all evaluated parameters. Differences were considered significant if P-value is lower than 0.05.

RESULTS AND DISCUSSION

EGCG content in green tea extract

EGCG content in green tea extracts are listed in Table 1. Green tea extracts with addition of guava powder had higher EGCG content than control sample (green tea only). The addition of 3 and 4 parts of guava powder significantly increased the total of EGCG in green tea extracts. Among the five formulas of green tea and guava, the combination of 2 parts of green tea and 3 parts of guava gave the highest EGCG content (39.03 ± 3.65 mg/g). According to the scanning result of TLC plate (Figure 1), there was no EGCG peak in guava sample, indicated that green tea was the only one that contributed to the amount of EGCG detected.

Table 1. Content of *Epigallocatechin gallate* (EGCG) detected in combinations of green tea and guava extracts

Formula	EGCG content (mg/g)
GT:GV 2:0	28.39 ± 2.45[a]
GT:GV 2:1	31.41 ± 0.75[ab]
GT:GV 2:2	30.80 ± 0.49[a]
GT:GV 2:3	39.03 ± 3.65[c]
GT:GV 2:4	37.90 ± 3.30[bc]

Data are means ± standard deviation (n = 3). Figures with different letters are statistically different at P < 0.05 as processed by the Tukey HSD test.

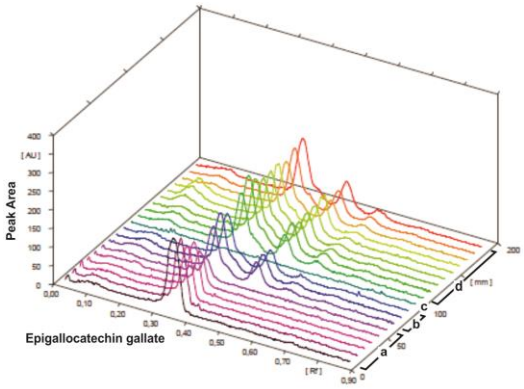

Figure 1. Scanned TLC plate showing the peaks obtained from *(-)-Epigallocatechin gallate* (EGCG) standard (a), green tea extract (b), guava extract (c), green tea added with guava extract (d). All extracts were obtained using liquid-liquid extraction

Lin & Yin (2012) reported that no epicatechin detected in guava aqueous extract. Rojas-Garbanzo *et al.* (2017) analyzed guava fruit using UHPLC-DAD-MS/MS and reported that it contained epigallocatechin gallate and other catechin compounds but due to overlapping peaks and low intensities, the quantification of those compounds was not achieved. EGCG in guava was not detected in this study probably due to its small concentration.

The increase of EGCG content in extracts added with guava fruit shown in Table 1 was likely due to the ability of guava to reduce the pH of the solution from

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6.08 to 4.65. EGCG is known to less stable in solution form. It was noted that pH affected the stability of EGCG. At neutral and alkaline environment (pH > 5.5), EGCG could undergo auto-oxidation, yielding dimers such as theasinensin A. Meanwhile acidic environment (pH = 2 - 5.5) enhanced the stability of EGCG (Krupkova *et al*., 2016). Bazinet *et al.* (2010) previously reported in their work that adjusting the acidity of green tea solution to pH 3.8 - 4.0 by lemon juice addition increased the stability of EGCG during long-term storage.

It is also speculated that ascorbic acid from guava increased the stability of EGCG. Ascorbic acid was reported significantly stabilized catechins in alkaline solution. Since ascorbic acid is known as antioxidant, it may work as reductant to protect the catechin in green tea. Another assumption, ascorbic acid may decrease the oxidation of catechin in green tea by reducing the concentration of dissolved oxygen in solution (Chen *et al.*, 1998). This was in agreement with the reports by Fangueiro *et al*. (2014), who examined the stability of EGCG which was spiked with ascorbic acid and found that ascorbic acid could reduce the degradation of EGCG by 80%.

The ability of fruits on stabilizing EGCG is consistent with previous reports explained that pomegranate syrup stabilized green tea catechins content during 15 days storage (Dhaouadi *et al.*, 2016). The high level of sugar in pomegranate syrup was suggested made a role in maintaining catechins stability. The previous study by Xu *et al*. (2014) explained the effect of viscosity in maintaining the stability of green tea infusion. It was reported that higher viscosity improved the stability of green tea infusion while being kept in cool terperature.

Antioxidant activities of green tea extracts

DPPH is a stable radical compound that absorbs visible light at a wavelength of 515 - 517 nm (Apak *et al*., 2013). The DPPH test measures the antioxidant properties of a compound based on their ability to capture DPPH radicals. DPPH free radicals can accept hydrogen atoms or electrons from antioxidants and form non-radical DPPH molecules. The antioxidant activity of green tea was observed from the change in color of DPPH solution which was originally dark purple to pale yellow. Table 2 shows the DPPH scavenging activity of green tea with and without the addition of guava.

Table 2. Antioxidant activities of the combination of green tea and guava extracts

Formula	Antioxidant activity (%)						IC_{50} (ppm)
	C = 500 ppm	1000 ppm	1500 ppm	2000 ppm	2500 ppm	3000 ppm	
GTGV 2:0	14.77 ± 0.04	26.98 ± 0.04	35.08 ± 0.04	45.84 ± 0.01	43.02 ± 0.06	59.63 ± 0.10	2356.46 ± 3.16[a]
GTGV 2:1	15.60 ± 0.04	26.19 ± 0.06	37.40 ± 0.04	47.58 ± 0.05	57.34 ± 0.32	64.76 ± 0.07	2176.88 ± 6.54[b]
GTGV 2:2	16.63 ± 0.01	24.90 ± 0.05	32.98 ± 0.10	43.37 ± 0.03	51.50 ± 0.05	61.58 ± 0.02	2388.63 ± 1.28[c]
GTGV 2:3	28.05 ± 0.04	30.37 ± 0.02	40.47 ± 0.03	52.96 ± 0.02	60.39 ± 0.03	69.82 ± 0.01	1917.32 ± 1.75[d]
GTGV 2:4	16.40 ± 0.05	27.49 ± 0.02	40.98 ± 0.01	51.88 ± 0.04	59.04 ± 0.01	69.07 ± 0.02	2025.87 ± 2.19[e]

Data are means ± standard deviation from three replicates. For IC_{50} column, figure followed by different letter have statistical difference at $P < 0.05$ as analyzed by the Tukey HSD test.

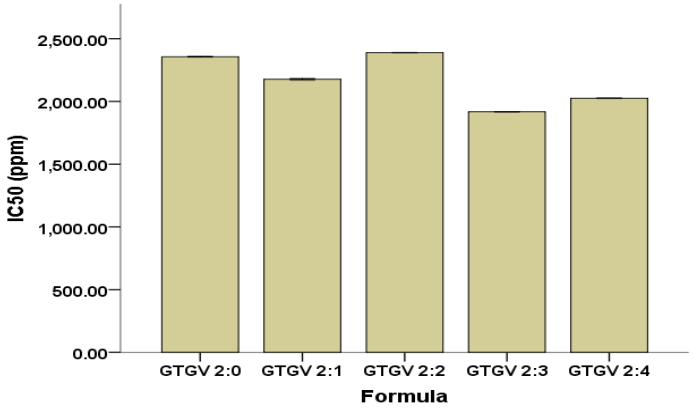

Figure 2. Antioxidant activity of green tea extracts with and without guava addition expressed as IC_{50}

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The radical scavenging activity (RSA) for green tea extracts at different levels indicate a higher concentration of sample provided higher scavenging activity. This result is due to the increasing number of EGCGs that can donate their hydrogen atoms to DPPH radicals. It was reported that hydroxyl groups in B ring and a gallate esterified at C ring of EGCG structure contribute to the antioxidant capacity of EGCG (Krupkova *et al*., 2016).

The effect of guava addition to green tea infusion was shown in Figure 2. Green tea infusion with the presence of guava had significant ($P < 0.05$) lower IC_{50} value than green tea infusion alone. Formula 4 which contained 2 parts of green tea and 3 parts of guava had the smallest IC_{50} value (1917.32 ± 1.75 ppm). The value of IC_{50} represents the amount of sample needed to scavenge 50% of DPPH radicals. The lower IC_{50} value means the higher DPPH radical scavenging activity. Obtained results suggested that the addition of guava powder was able to significantly strengthen the antioxidant activity of green tea up to 19%.

The antioxidant activity of the combination formulas was also affected by the presence of other flavonoids in green tea and guava. Green tea was reported to contain other flavonoids besides EGCG, such as catechin, epicatechin, quercetin-3-rutinoside, kaempferol-3-rutinoside, myricetin-3-glucoside, hesperetin-7-rutinoside, and naringenin-7-rutinoside (Pekal *et al*., 2012). It has been found that guava also has antioxidant activity due to its flavonoid and phenolic contents (Yan *et al*., 2006; Musa *et al*., 2015). The flavonoids detected in the pink guava were kaempferol, isorhamnetin, myricetin, luteolin, and quercetin (Musa *et al*., 2015). Another study by Nunes *et al*. (2016) examined guava using HPLC-DAD and the phenolic compounds of guava were myricetin, quercetin, quercetin-3-O-rutinoside, naringenin, 3-4-dihydroxybenzoic acid, 2-hydroxybenzoic acid, benzoic acid, gallic acid, syringic acid, 3-hydroxycinnamic acid, ferulic acid, rosmarinic acid, and 3,4-dihidroxyphenylacetic acid. Lin & Yin (2012) also reported that guava aqueous extract contained several compounds, such as ascorbic acid, rutin, quercetin, rosmarinic acid, myricetin, caffeic acid, naringenin, coumaric acid, ferulic acid, and ellagic acid.

CONCLUSION

In general, the addition of guava powder significantly increased the amount of EGCG in green tea extracts. Among all the samples, formula 4 (2 parts of green tea and 3 parts of guava) gave the highest EGCG content (37.5% higher than the control sample). The ability of guava to reduce the pH of the solution and the presence of ascorbic acid were considered able to maintain the stability of EGCG. The presence of guava also enhanced the antioxidant activity of green tea solution up to 19%. The results were likely due to the combined effect of flavonoids contained in green tea and guava.

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