CATECHIN CONTENTS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF DIFFERENT TYPES OF INDONESIAN TEA (*CAMELLIA SINENSIS*)

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Abstract

Tea is one of the most popular beverages in the world. Produced from *Camellia sinensis* leaves, tea has been widely studied for its health benefits due to the content of essential metabolites. This study aimed to investigate the catechin contents, antioxidant, and antibacterial activities of Indonesian tea varieties, namely green tea, black tea, and white tea. Tea infusion was prepared by extracting 1 g of each sample into 10 mL of distilled water and incubated at 80 °C for 60 min. The catechin and epigallocatechin gallate (EGCG) contents of tea extracts were determined using high-performance liquid chromatography (HPLC). Antioxidant activity was measured using the free radical method with 2,2-diphenyl-1-pycrylhidrazyl (DPPH), while antimicrobial activity was assessed using paper disc diffusion assay. The results indicated that green tea had the highest contents of catechin (646 ± 17.14 mg/L) and EGCG (997.8 ± 36.72 mg/L), and antioxidant activity with IC_{50} of 5.65 µg/mL. Furthermore, green tea and white tea extracts showed inhibitory activity against Gram-positive bacteria such as *Micrococcus luteus, Bacillus subtilis*, and *Staphylococcus aureus* whereas black tea had no activity against all bacterial strains tested. Generally, we concluded that white tea and green tea contributed to the higher content of catechins and exhibited strong antioxidant and antibacterial properties.

Keywords: Antibacterial, *Camellia sinensis*, Catechin, EGCG, Tea

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Introduction

The tea plant (*Camellia sinensis*) is one of the most important crops and commodities in the world. It is an evergreen perennial plant that is widely grown and cultivated in some tropical and subtropical countries of Asia (China, Japan, India, Indonesia), Africa (Kenya, Malawi), and Latin America (Sharma *et al.*, 2009; Wang *et al*., 2019; Zhou *et al.*, 2017). In Indonesia, tea has become an important commodity that has been exported to several countries. Recently, Indonesia stands as the seventh world's biggest tea producer and contributes to approximately 2.3% of total tea production worldwide (Rezamela *et al*., 2020). In the last decade (2009-2018), Indonesian tea export showed increasing in demand. This positive trend is expected to encourage social welfare and the national economy (Khaliqi *et al.*, 2020). Based on these facts, the effort to monitor the quality of Indonesian tea from cultivation, production, packaging, and product storage becomes important. Thus, Indonesian tea products can remain competitive in the global market.

As an aqueous product extracted from *C. sinensis*, tea has become one of the most popular beverages due to its pleasant flavor, taste, and bioactive ingredients for health (Ikka *et al.*, 2018; Li *et al.*, 2015). Previous studies have reported that tea leaves contain important bioactive compounds such as polyphenols, alkaloids, caffeine, and theanine. Typically, the major polyphenols of the tea leaves are catechin

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(C), epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin gallate (EGCG) (Cardoso *et al.*, 2020; Steinmann *et al.*, 2013). Bioactive compounds play important roles in the tea quality and contribute to human health benefits (Park *et al.*, 2004). Several studies have shown that tea polyphenols have benefits in pharmacological and medical properties such as antioxidant, antimicrobial, antidiabetic, anti-inflammatory, anticarcinogenic, and anti-obesity effects (Bansal *et al.*, 2012; El-Shahawi *et al.*, 2012). Furthermore, tea catechins provide strong antioxidant activity, effective scavengers, and excellent electron donors to prevent the damage of cells caused by oxidative stress. In addition, tea catechins particularly EGCG, have antibacterial activity against both Gram-positive and Gram-negative bacteria by inhibiting their growth (Almajano *et al.*, 2008; Bancirova, 2010).

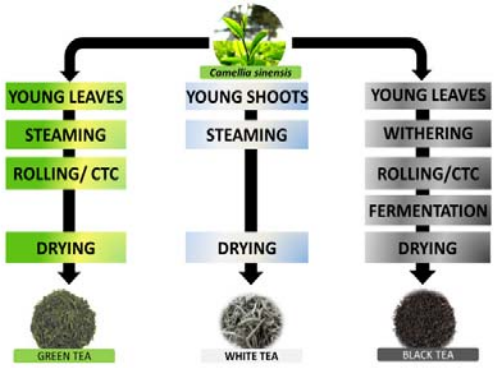

Figure 1. The different approaches of the tea manufacturing process in green tea, white tea, and black tea.

According to the method of processing, teas are classified into three main categories based on their level of oxidation or fermentation degrees: non-fermented (green and white), partially fermented (oolong), and fully fermented (black and Pu-erh) (Lee *et al.*, 2014). For non-fermented tea produced from fresh leaves such as green tea and white tea, the primary polyphenol contents are mostly catechins (Senanayake, 2013). In contrast, the oxidation of polyphenols occurred in black tea during the fermentation process. Consequently, the process leads to the transformation of catechins into theaflavins and thearubigins, which are the main polyphenols in black tea (Tanaka & Kouno, 2003). As the extent of the fermentation process is increased, antioxidant and antibacterial activities decrease. This implies stronger activity in non-fermented tea than fermented tea (Tiwari *et al.*, 2005).

In order to monitor and evaluate the quality of Indonesian tea products originated from Gambung, West Java, such as green tea, black tea, and white tea, the investigation of their catechin and EGCG contents as well as antioxidant and antibacterial activities should be conducted. Moreover, understanding biochemical compounds in different types of tea is necessary to further develop tea production in Indonesia.

Materials and Methods

Materials.

Three types of Indonesian tea namely black tea, green tea, and white tea were obtained from the Research Institute of Tea and Cinchona in Gambung, Bandung Regency, West Java, Indonesia. All samples were produced from Assamica variety leaves following the standard procedure of tea production. Catechin (C) and epigallocatechin gallate (EGCG) were purchased from Wako Pure Chemical Industries, whereas acetonitrile was purchased from Sigma. All reagents used in this study were HPLC grade. For antibacterial activity assay, six bacterial strains used were *Micrococcus luteus* BTCC B-552, *Bacillus subtilis* BTCC B-612, *Staphylococcus aureus* BTCC B-611, *Pseudomonas aeruginosa* ATCC 9027, *Mycobacterium smegmatis*, and *Escherichia coli* BTCC B-614. These bacteria were provided by the Laboratory of Applied Microbiology, Research Center of Biotechnology, Indonesian Institute of Sciences, Bogor, West Java.

Tea Sample Preparation.

Tea samples were extracted according to Vuong *et al.* (2011) method with a slight modification. Tea infusion was prepared by weighing 1 g of tea sample in a conical tube and adding 10 mL of distilled water. It was then mixed using a vortex and incubated at 80 °C for 60 min using a water bath. After cooling at room temperature, tea extract was centrifuged at 10,000 rpm, 20 °C for 5 min. The supernatant was directly used for HPLC analysis. Meanwhile, for antioxidant and antimicrobial

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assay, the supernatant was concentrated using a freeze dryer.

Determination of Catechin and EGCG Contents Using HPLC.

Tea extract was placed and filtrated in an HPLC vial with a 0.45 µm filter. The catechin and EGCG contents were analyzed using an Agilent Technology system. The HPLC conditions were set as follows: Agilent Porocel EC C-18 (150 mm) phase column was maintained at 50 °C and detection was performed at 210 nm with an ultraviolet diode-array detector (DAD). The mobile phase used was ultrapure water HPLC grade (A) and acetonitrile (B) with a ratio of 9:1 (v/v). Twenty microliters of each sample were injected with a run time of 25 min and a flow rate of 1 mL per min for each sample. To identify the catechin contents in each sample, catechin and EGCG were used as standards for their retention time. All standards were diluted in ultrapure water and prepared at different concentrations of 12.5; 25; 50; 100; and 200 mg/L. The quantification of catechins was measured using a regression equation from a serial concentration of standards.

Determination of Antioxidant Activity using DPPH Assay.

Antioxidant activity was performed using a free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging test (Pyrzynska & Pekal, 2013). Briefly, to measure the radical scavenging ability, 50 µL of each extract at serial concentrations: 2, 4, 6, 8, 10, and 12 µg/mL was added into 50 µL of 0.1 mM DPPH in methanol and homogenized using a vortex. The mixture was incubated in the dark at room temperature (27 °C) for 30 min. Ascorbic acid (Vitamin C) was used as a positive control, while the DPPH solution was used as a blank. The discoloration was measured at the wavelength of 517 nm using a UV-Vis spectrophotometer. The DPPH radical scavenging activity was calculated using the formula below:

$$DPPH \text{ scavenging } (\%) = \frac{A\ blank - A\ sample}{A\ blank}\ x\ 100$$

Where A_{blank} was the absorbance of the blank (sample solution or water) and A_{sample} was the absorbance of the test sample. The IC_{50} value (x) was defined as the concentration of the sample required to scavenge 50% of free radicals (y) and indicated antioxidant capacity. The value was calculated using linear regression analysis (Mohamed *et al*., 2013):

$$y = ax + b$$

Determination of Antibacterial Activity Using Paper Disc Diffusion Assay.

A total of six bacterial strains were cultured in Nutrient Broth (NB) media and incubated at 30 °C and 150 rpm. After 24 h of incubation, their turbidity was measured using a spectrophotometer at 560 nm and then adjusted to the turbidity standard (Absorbance = 0.9). All bacterial strains were grown in Mueller Hinton Agar (MHA) media. The MHA was prepared in two layers: half ingredient of the upper layer medium was to culture the bacteria, whereas the complete ingredient of the bottom layer was only the sterile medium. A disc diffusion method was used for this assay. Filter paper discs with a 6 mm diameter were prepared and sterilized. A 10 µL of each extract (25 µg/mL) was added to the paper disc. The impregnated discs with extracts were then placed on the agar surface and incubated at 4 °C for 30 min. Furthermore, Chloramphenicol (25 µg/mL) was used as a positive control while the solvent was used as a negative control. The antibacterial activity was expressed by showing a clear zone around the paper disc. The diameter (D) of the inhibitory zone (mm) was measured using the equation below:

$$Inhibitory\ zone\ (mm) = D\ paper - D\ inhibition$$

Data Analysis.

All experiments were performed at least in duplicate. The catechin and EGCG contents, the percentage of DPPH scavenging, and the IC_{50} value presented in the results were expressed as the mean and standard deviation (± SD).

Results

Catechin and EGCG Contents.

The different tea processing approaches resulted in various catechin and EGCG contents in the fermented tea (black tea) and non-fermented tea (green tea and white tea). The catechin and EGCG contents from samples were obtained based on the quantification using linear regression from the respective standard. The calibration curve of the respective reference standard is shown in Figure 2.

Annales Bogorienses Vol. 24, No. 2, 2020
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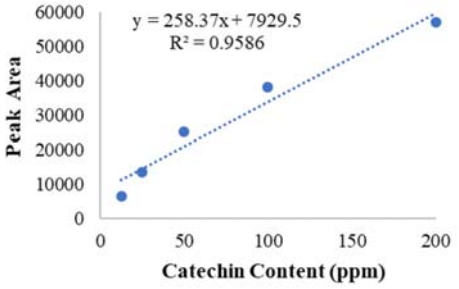
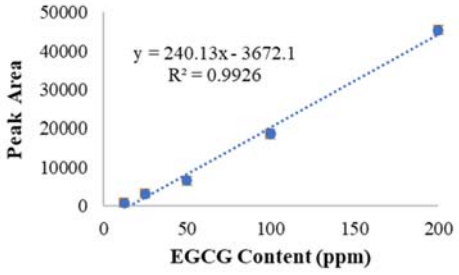


(A)



(B)

Figure 2. Calibration curves of reference standard: (A) Catechin and (B) Epigallocatechin gallate (EGCG).

In this current study, the hot water extraction method was applied since it becomes a simple way of brewing tea. As depicted in Figure 3, by using the hot water extraction, green tea had the greatest amount of catechin and EGCG contents (615.4 and 945.5 ppm, respectively), followed by white tea (427.4 and 714.7 ppm) and black tea (44.03 and 108.5 ppm). These results indicated that EGCG, which is the most dominant catechin derivate, showed a much higher content than catechin present in the respective sample tested.

Based on the available data, catechins are abundant in green tea and white tea. In contrast, catechins in black tea become decreased due to the transformation of catechins into theaflavins and thearubigins during the fermentation process (Menet *et al.*, 2004). Generally, these findings revealed that non-fermented tea (green tea and white tea) was an excellent source of catechin and EGCG compared to fermented tea (black tea).

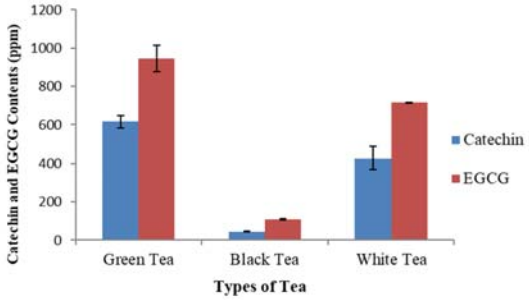


Figure 3. Catechin and EGCG contents in different types of tea.

Antioxidant Activity.

The scavenging ability of DPPH free radicals of tea extracts is presented in Figure 5. The DPPH was used as stable free radicals to examine the antioxidant activity of 10 µg/mL of each sample. According to the results, green tea had the highest percentage of DPPH scavenging ability (67.3%), indicating the most potent antioxidant activity among the other samples tested (white tea at 47.9% and black tea at 28.9%).

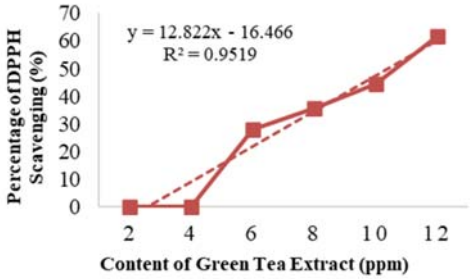


Figure 4. Correlation curve between the concentration of green tea extract and the percentage of DPPH scavenging.

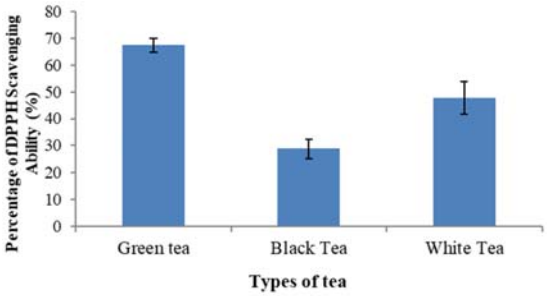


Figure 5. The percentage of DPPH scavenging ability in different extracts of tea (10 µg/mL).

To determine the concentration of tea extract required to scavenge 50% of DPPH free

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radicals, the IC_{50} value was measured. The IC_{50} value is inversely proportional to the antioxidant activity of the sample. That means the lower the IC_{50} value, the higher the antioxidant activity.

Table 1. IC_{50} value in different tea samples and vitamin C

Sample	IC_{50} Value (µg/mL)
Vitamin C	2.74 ± 0.3
Black tea	43.5 ± 7.3
Green tea	5.65 ± 1.4
White tea	7.93 ± 2.2

As shown in Table 1, the sample's rank from the lowest to the highest IC_{50} value was in the order of green tea < white tea < black tea. This result means in terms of antioxidant activity, green tea > white tea > black tea, but all samples had lower antioxidant activity than vitamin C. The vitamin C had only a slight difference in antioxidant activity over green tea and white tea, referring to the IC_{50} value.

Antibacterial Activity.

Three varieties of tea were investigated for their potential antibacterial activity against six pathogenic bacteria. Chloramphenicol was used for this assay due to its potent activity as a broad-spectrum antibiotic and antibacterial against both Gram-positive and negative bacteria. As presented in Table 2, green tea and white tea extracts showed their activity to inhibit the growth of Gram-positive bacteria, but no inhibitory effects were observed against all bacteria tested for black tea extract. The strain *B. subtilis* was the most susceptible to white tea extract (8.0 mm), whereas *M. luteus* showed the greatest sensitivity to green tea extract (11.35 mm). The greater diameter of inhibition, the higher the inhibitory activity.

Table 2. Antibacterial activities of different tea samples against Gram-positive bacteria

Sample (25 µg/mL)	Zone of inhibition (mm)		
	B. subtilis	***S. aureus***	***M. luteus***
Chloramphenicol (Positive control)	18.25	20	35
Black tea	0	0	0
Green tea	2.65	9.025	11.35
White tea	8.0	4,875	2.85

Table 3. Antibacterial activities of different tea samples against Gram-negative bacteria

Sample (25 µg/mL)	Zone of inhibition (mm)		
	E. coli	***M. smegmatis***	***P. aeruginosa***
Chloramphenicol (Positive control)	26.5	22.34	22.5
Black tea	0	0	0
Green tea	0	0	0
White tea	0	0	0

Data shown in Table 3 revealed that black tea, green tea, and white tea had no antibacterial activity against *E. coli*, *M. smegmatis*, and *P. aeruginosa.* It seemed that hot water tea extract was not quite effective inhibiting the growth of Gram-negative bacteria.

Discussion

This study highlighted the contents of catechin and its derivate, EGCG, in black tea, green tea, and white tea as well as their potential as antioxidants and antibacterials. The amount of catechin and EGCG in every tea product depends primarily on its variety of the plant, cultivation conditions, harvesting time, manufacturing process, and brewing temperature (Saklar *et al*., 2015; Wei *et al.*, 2011). Since the samples were collected from the same variety (Assamica) at identical conditions, all those parameter's effects could be eliminated, except for the manufacturing process. Compared to non-fermented tea (e.g. green tea and white tea), black tea has much lower catechin and EGCG contents due to the oxidation of polyphenol compounds during the fermentation process. In a related study, Yoshida *et al.* (1999) has demonstrated that EGCG was the most abundant compound and represented 50-80% of the total catechins in green tea. The use of hot water extraction effectively produced the highest yield of tea extracts compared to methanol and ethyl acetate extraction. The high temperature can decrease the polarity of water but increase its capability to dissolve less polar compounds. Thus, the efficiency of the extraction process can be reached. In addition, the high temperature of the water also reduces its viscosity and surface tension, therefore the diffusion rate increases

Annales Bogorienses Vol. 24, No. 2, 2020
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during the extraction process (Chan *et al*., 2011).

The presence of catechin and EGCG in the tea sample contributed to the positive impact of antioxidant activity. In this study, the antioxidant activity was higher in non-fermented tea than fermented tea. Due to their three adjacent hydroxyl (OH) groups on the B-ring particularly EGCG in green tea and white tea are much higher than those in black tea. The compound of EGCG is responsible for scavenging free radicals (Almajano *et al.*, 2008; Chan *et al.*, 2011). In addition, the antioxidant property of black tea has been provided by thearubigins and theaflavins. Several earlier studies have shown that green tea has more potent antioxidant properties than black tea (Nibir *et al.,* 2017; Kaur *et al.,* 2015). Therefore, the higher the EGCG content in the tea sample, the higher the antioxidant activity.

As reported in the previous study, black tea and green tea extracts could inhibit the growth of both Gram-positive and Gram-negative bacteria (Bancirova, 2010; Nibir *et al.*, 2017). However, the results of this study demonstrated that white tea and green tea exhibited no antibacterial activity against Gram-negative bacteria tested, whereas black tea showed no antibacterial activity against both Gram-positive and Gram-negative bacteria tested. A similar finding has also reported that green tea has inhibitory effects against Gram-positive but not Gram-negative bacteria. Tea extracts are not effective against *P. aeruginosa* and *E. coli* (Chan *et al.,* 2011). Some conditions such as the different variety of *C. sinensis*, types of extraction solvents, and bacterial strains might imply and contribute to the differences in the results. Most Indonesian teas are produced from assamica variety instead of sinensis variety. Soil and weather conditions in Indonesia, which are different from subtropical countries also contribute to tea quality disparity.

In conclusion, this research is a preliminary study to reveal the contents of catechin and EGCG from black tea, green tea, and white tea, and their antioxidant and antimicrobial activities. The different tea processing methods may result in the disparity of catechin contents, the antioxidant, and antimicrobial activity among all these types of Indonesian teas. The present study suggests that green tea and white tea may contribute to the higher content of catechin and EGCG and can be potent to provide strong antioxidant and antibacterial activities. Therefore, further studies are necessary to thoroughly investigate the different tea polyphenols in fermented tea and non-fermented tea.

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References

Almajano, M. P., Carbo R., Jimenez, J. A., & Gordon, M. H. (2008). Antioxidant and antimicrobial activities of tea infusions. *Food Chemistry, 108,* 55-63. doi:10.1016/j.foodchem.2007.10.040

Bancirova, M. (2010). Comparison of the antioxidant capacity and the antimicrobial activity of black and green tea. *Food Research International, 43,* 1379-1382. doi:10.1016/j.foodres.2010.04.020

Bansal, S., Syan, N., & Mathur, P. (2012). Pharmacological profile of green tea and its polyphenols: A review. *Medicinal Chemistry Research, 21,* 3347-3360. doi:10.1007/s00044-011-9800-4

Cardoso, R. R., Neto, R. O., Dos Santos D'Almeida, C. T., Nascimento, C. T., Pressete, C. G., Azevedo, L., Martino, H. S. D., Cameron, L. C., Ferreira, M. S. L., & Barros, F. A. R. (2020). Kombuchas from green and black teas have different phenolic profile, which impacts their antioxidant capacities, antibacterial and antiproliferative activities. *Food Research International, 128*.

Chan, E. W., Soh, E. Y., Tie, P. P., & Law, Y. P. (2011). Antioxidant and antibacterial properties of green, black, and herbal teas of *Camellia sinensis*. *Pharmacognosy Research, 3,* 266-272.

El-Shahawi, M. S., Hamza, A., Bahaffi, S. O., Al-Sibaai, A. A., & Abduljabbar, T. N. (2012). Analysis of some selected catechins and caffeine in green tea by high-performance liquid chromatography. *Food Chemistry, 134*, 2268-2275. doi:10.1016/j.foodchem.2012.03.039

Ikka, T., Yamashita, H., Kurita, I., Tanaka, Y., Taniguchi, F., Ogino, A., *et al*. (2018). Quantitative validation of nicotine production in tea (*Camellia sinensis* L.). *PLoS ONE, 13*(4). https://doi.org/10.1371/journal.pone.0195422

Kaur, A., Kaur, M., Kaur, P., Kaur, H., Kaur, S., & Kaur, K. (2015). Estimation and comparison of total phenolic and total antioxidants in green tea and black tea. *Global Journal of Bioscience and Biotechnology, 4*(1), 116-120.

Khaliqi, M., Gurning, H. R. H., Novanda, R. R., & Simamora, O. N. (2020). Competitiveness Indonesia tea in international market. *IOP Conference Series: Earth and Environmental Sciences, 454*.

Lee, L. S., Kim, S. H., Kim, Y. B., & Kim, Y. C. (2014). Quantitative analysis of major constituents in green tea with different plucking periods and their antioxidant activity. *Molecules, 19,* 9173-9186.

Li, Q., Li, J., Liu, S., Huang, J., Lin, H., Wang, K., Cheng, X., & Liu, Z. (2015). A comparative proteomic analysis of the buds and the young expanding leaves of the tea plant (*Camellia sinensis* L.). *International Journal of Molecular Sciences*, *16*(6), 14007-14038. https://doi.org/10.3390/ijms160614007

Menet, M. C., Sang, S., Yang, C. S., Ho, C. T., & Rosen, R. T. (2004). Analysis of theaflavins and thearubigins from black tea extract to MALDI-TOF mass spectrometry. *Journal of Agricultural and Food Chemistry, 52(9)*, 2455-2461. doi: 10.1021/jf035427e

Mohamed, A. A., Ali, S. I., & El – Baz, A. F. (2013). Antioxidant and antibacterial activities of crude extracts and essential oils of *Syzygium cumini* leaves. *PLoS ONE, 8.* doi:10.1371/journal.pone.0060269

Nibir, Y., Sumit, A. F., Akhand, A. A., Ahsan, N., & Hossain. (2017). Comparative assesment of total polyphenols, antioxidant and antimicrobial activity of different tea varieties of Bangladesh. *Asian Pacific Journal of Tropical Biomedicine, 7 (4)*, 352-357. doi:10.1016/j.apjtb.2017.01.005

Park J. S., Kim J. B., Hahn B. S., Kim K. H., Ha S. H., & Kim Y. H. (2004). EST analysis of genes involved in secondary metabolism in *Camellia sinensis* (tea), using suppression subtractive hybridization. *Plant Science*, *166*, 953–961. doi: 10.1016/j.plantsci.2003.12.010

Pyrzynska, K. & Pekal, A. (2013). Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples. *Analytical Methods, 5,* 4288-4295. doi:10.1039/C3AY40367J

Rezamela, E., Syahrian, H. K., Wulansari, R., Prawira-Atmaja, M. I., Agustian, E., & Adilina, I. B. (2020). Polyphenols content of Indonesian tea slones on optimum manuring condition. *IOP Conference Series: Earth and Environmental Science, 439*.

Saklar, S., Ertas, E., Ozdemir, I. S., & Karadeniz, B. (2015). Effects of different brewing conditions on catechin content and sensory acceptance in Turkish green tea infusions. *Journal of Food Science and Technology*, *52*(10), 6639–6646. https://doi.org/10.1007/s13197-015-1746-y

Sharma, R. K., Bhardwaj, P. B., Negi, R., Mohapatra T., & Ahuja, P. S. (2009). Identification, characterization, and utilization of unigene derived microsatellite markers in tea (*Camellia sinensis* L.). *BMC Plant Biology, 9,* 1-24.

Senanayake, S. P. J. N. (2013). Green tea extract : chemistry, antioxidant properties, and food applications – A review. *Journal of Functional Foods, 5*(4), 1529–1541. https://doi.org/10.1016/j.jff.2013.08.011

Steinmann, J., Buer, J., Pietschmann, T., & Steinmann, E. (2013). Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea. *British Journal of Pharmacology, 168,* 1059–1073

Tanaka, T., & Kouno, I. (2003). Oxidation of tea catechins: chemical structures and reaction mechanism. *Food Science and Technology Research, 9*(2), 128–133. https://doi.org/10.3136/fstr.9.128

Tiwari, R. P., Bharti, S. K., Kaur, H. D., Dikshit, R. P., & Hoondal, G. S. (2005). Synergistic antimicrobial activity of tea and antibiotics. The *Indian Journal of Medical Research, 122,* 80-84.

Vuong, Q. V., Golding, J. B., Stathopoulos, C. E., Nguyen, M. H., & Roach, P. D. (2011). Optimizing conditions for the extraction of catechins from green tea using hot water. *Journal of Separation Science, 34,* 3099-3106. doi:10.1002/jssc.201000863

Wang, X., Liu, B. Y., Zhao, Q., Sun, X., Li, Y., Duan, Z., Miao, X., Luo, S., & Li, J. (2019). Genomic variance and transcriptional comparisons reveal the mechanisms of leaf color affecting palatability and stressed defense in tea plant. *Genes*, *10*(11), 929. https://doi.org/10.3390/genes10110929

Wei, K., Wang, L., Zhou, J., He, W., Zeng, J., Jiang, Y., & Cheng, H. (2011). Catechin contents in tea (*Camellia sinensis L.*) as affected by cultivar and environment and their relation to chlorophyll contents. *Food Chemistry., 125*, 44–48. doi: 10.1016/j.foodchem.2010.08.029.

Yoshida, Y., Kiso, M., & Goto, T. (1999). Efficiency of the extraction of catechins from green tea.

Food Chemistry, 67, 429-433. doi:10.1016/S0308-8146(99)00148-X

Zhou, Q., Chen, Z., Lee, J., Li, X., & Sun, W. (2017) Proteomic analysis of tea plants (*Camellia sinensis*) with purple young shoots during leaf development. *PLoS ONE, 12*(5). https://doi.org/10.1371/journal.pone.0177816