Potential of Black Tea (*Camellia Sinensis* (L.) O. Kuntze) Extract as Anti-oxidant and Skin Anti-aging

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Abstract: Background: Skin aging is characterized by features such as wrinkling, loss of elasticity, laxity, rough textured appearance, and phenotypic changes in cutaneous cells. Skin aging treatment usually with a synthetic compound with unknown side effects but with herbal such as black tea, these side effects will be minimalized. Objective: This research was conducted to evaluate the qualitative phytochemical screening assay, total phenolic and flavonoid contents, anti-oxidants, and skin anti-aging properties of black tea extract (BTE). Method: This qualitative phytochemical content using the Farnsworth modified method. Total phenol content was calculated using gallic acid equivalent (GAE), and total flavonoid content was calculated using quercetin equivalent (QE). The anti-oxidant properties using 2,2 diphenyl 1 picrylhydrazyl (DPPH), 2,2′-Azinobis (3- Ethylbenzthiazoline-6-Sulfonate) (ABTS), hydrogen peroxide (H2O2) scavenging activities. The anti-aging properties were assayed using elastase and collagenase inhibition activities. Results: BTE contained terpenoids, triterpenoids, phenols, flavonoids, tannins. BTE contained phenol 52.81 µg GAE/mg, flavonoids 10.96 QE/mg. The IC50 value of DPPH, ABTS, H2O2 scavenging activities was 15.29; 88.18; 17.21 µg/ml respectively. The IC50 value of elastase and collagenase inhibition was 31.34; 123.74 µg/ml respectively. Conclusion: BTE has highly active and active anti-oxidant and is also less active and moderately active in skin anti-aging activities.

1 INTRODUCTION

Aging is a normal physiological process experienced by all creatures. Skin is the organ that is exposed to the outer environment so skin suffers from both intrinsic and extrinsic aging factors. Skin aging can be characterized by wrinkling, loss of elasticity, laxity, and rough-textured appearance (Zhang & Duan, 2018). There are two kinds of factors inducing skin aging intrinsic and extrinsic factors. Intrinsic factors are mainly due to genetic and metabolic factors (Mancini et al., 2014). Collagenase is the enzyme to cleaving native collagen under the physiological condition *in vivo* and *in vitro* (Holmbeck & Birkedal Hansen, 2013; Widowati et al., 2016; 2017; 2018a, 2018b). While elastin plays a role in maintaining the elasticity of the skin that can be degraded by enzymes elastase. While the factors extrinsic including, stress, lifestyles such as smoking and drinking alcohol, exposure to sun or UV rays. Premature aging is most often caused by air pollution and photoaging or UV light. The effects of

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photoaging exposure in the long term will result in an increased risk of premature aging caused by Reactive Oxygen Species (ROS) which can affect collagenase and elastase enzymes and levels of anti-oxidants in the body. That will make the appearance of wrinkles and dark stains on the skin arise. (Widowati et al., 2016; 2017; 2018a, 2018b).

To ward off free radicals in the body, humans can produce anti-oxidant enzymes, e.g., glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD) (Yadav et al., 2016), but the amount is still less effective in overcoming oxidative stress that happens to the body. Many people consume anti-oxidants and skin anti-aging synthesis as cosmetics or creams, but over long periods can cause side effects such as hyperpigmentation or malignancy of the skin. So it is sought alternatives from natural ingredients which have anti-oxidant and skin anti-aging activities such as pineapple (Jusri, 2019), dragon fruit (Liana, et al., 2019), jasmine flowers (Widowati et al., 2018a), white rice (Widowati et al., 2016), rosella flowers (Widowati et al., 2017), and black tea extract have activity anti-oxidants (Widowati et al., 2015). Tea has a bioactive component, such as polyphenols. In general, the classification of polyphenols exists 2, namely phenolic acids and flavonoids (Sudaryat et al., 2015). The function of flavonoids is to protect the body from damage caused by ROS, inhibit degenerative diseases, and inhibit the activity of lipid peroxidase (Sudaryat et al., 2015). Tea also contains theophylline, tannins, vitamin B complex, C, E, K.12. The black tea contains bioactive compounds alkaloids, flavonoids and tannins, phenols, saponins, and steroids that are thought to have anti-oxidant activity, anti-collagenase, and anti-elastase (Sudaryat et al., 2015, Widowati et al., 2015).

This research was conducted to find out the content of various compounds phytochemicals of black tea extract obtained commercially manufacture. The study also measured anti-oxidant activity including H_2O_2, DPPH, and ABTS scavenging activity, and skin anti-aging activities including anti-collagenase and anti-elastase of black tea extract (BTE). This research was conducted for qualified cosmetic preparation to use certified extract based on good manufacture practice (GMP).

2 MATERIALS AND METHODS

2.1 Sample Preparation

BTE was obtained from Indesso (SP-766-3). The BTE using water solvent and followed by the spray drying process, moisture content 7.0%, turbidity ≤35 FAU, polyphenol content ≥ 15%, soluble in water, ingredient: BTE and maltodextrin (Certificate of Analysis by Indesso, Jakarta, Indonesia).

2.2 Phytochemical Analysis

Phytochemical analysis was used to determining the secondary metabolite content of BTE. The phytochemical analysis in this research consisted of flavonoid, saponin, phenolic, tannin, alkaloid, steroid/triterpenoid, and terpenoid content of BTE.

2.2.1 Flavonoid Content

A 10 mg extract of BTE dissolved in HCl 2 N in the reaction tube. In the mixture, Mg/Zn was added sufficiently, then was heated for 5-10 minutes, cooled down filtered, and added 1 ml of amyl-alcohol. If red/orange color formed then the sample contained flavonoid (Widowati et al., 2018a; Prahastuti et al., 2019; Prahastuti et al., 2020).

2.2.2 Saponin Content

A 10 mg of BTE dissolved in ddH2O in the reaction tube. The sample heated until boiling for 5 minutes then filtered and shake strongly before added HCl 1 N. If the bubble still formed and still exist after HCL 1 N was added, then the sample contained saponin (Widowati et al., 2018a; Prahastuti et al., 2019; Prahastuti et al., 2020).

2.2.3 Phenolic Content

A 10 mg of BTE dissolved in 5 ml ddH_2O. Briefly 500 µl $FeCl_3$ 1% added into mixture. If green/red/purple/blue/black color formed, the sample contained phenol (Widowati et al., 2018; Prahastuti et al., 2019; Prahastuti et al., 2020).

2.2.4 Tannin Content

A 10 mg of BTE dissolved in 2 ml HCl 2N in the reaction tube. The mixture was heated in the water bath for 30 minutes, cooled down, then 500 µl of amyl alcohol was added. If there was an orange/red color in the amyl-alcohol layer, then the sample contained tannin (Widowati et al., 2018; Prahastuti et al., 2019; Prahastuti et al., 2020).

2.2.5 Alkaloid Content

A 10 mg of BTE extract dissolved in 5 ml ddH_2O

and evaporated in the water bath. The residue from the evaporation dissolved in 5 ml HCl 2N. Then the mixture is divided into two reaction tubes. The first tube added 3 drops of HCl 2N as a blank. The mixture from the second tube took 1 drop into the dropping plate, then 3 drops of Dragendorff mixture were added. If the orange solid formed, then the sample contained alkaloids (Widowati et al., 2018a; Prahastuti et al., 2019; Prahastuti et al., 2020).

2.2.6 Steroid/Triterpenoid Content

Glacial acetic acid is added into 10 mg BTE in the dropping plate, let the mixture for 10-15 minutes. In the mixture added 1 drop of concentrated H2SO4. If there was greenish-blue color formed then the sample contained steroid, but if there was a purple/red/orange color formed then the sample contained triterpenoid (Widowati et al., 2018a; Prahastuti et al., 2019; Prahastuti et al., 2020).

2.2.7 Terpenoid Content

Vanillin was added into 10 mg BTE sufficiently into the dropping plate. 1 drop of concentrated H2SO4 was added then homogenized. If there was a purple color formed, then the sample contained terpenoid (Widowati et al., 2018a; Prahastuti et al., 2019; Prahastuti et al., 2020).

2.3 Total Phenolic Content

A 15 µl of a sample (BTE and gallic acid) was loaded into the sample and blank wells. Briefly, 75 µl Folin-Ciocalteu 10% reagent was added into sample wells. 60 µl Na2CO3 7.5% added to sample wells. Into the blank well, 150 µl DMSO 10% was added then incubated for 10 minutes at 50oC. Absorbance was measured with a microplate reader at λ = 760 nm. Gallic acid (GA) is used as a standard to determine the phenolic concentration (Widowati et al., 2018a; Prahastuti et al., 2019; Prahastuti et al., 2020).

$$\text{Linear standard equation: } y = ax + b \quad (1)$$

$$\text{Total Phenol} = \frac{Sample\ Absorbance - b}{a} \quad (2)$$

2.4 Total Flavonoid Content

Briefly, 75 µl of a sample (BTE and Quercetin) added to the sample and blank wells. Amount 75 µl AlCl3 2% (in glacial acetic acid 5% and methanol) was added into sample wells. Into the blank well, 75 µl DMSO was added. Absorbance was measured with a microplate reader at λ = 415 nm. Total flavonoid calculated with the standard linear equation of Quercetin (Widowati et al., 2018a; Prahastuti et al., 2019; Prahastuti et al., 2020).

$$\text{Linear standard equation: } y = ax + b \quad (3)$$

$$\text{Total Flavonoid} = \frac{Sample\ Absorbance - b}{a} \quad (4)$$

2.5 H2o2 Scavenging Activity

Method to determine the activity of H2O2 scavenging based on the modified method by Prahastuti et al. (2019 and (2020). Each sample well was contained 60 µL of BTE, 12 µL of FeH8N2O8S2 1 mM, and 3 µL of H2O2 5 mM. The mixture that contains 12 µL of FeH8N2O8S2 and 63 µL of DMSO was used as the negative control, while the mixture that contains 60 µL of BTE and 90 µL of DMSO were used as the blank solution. After H2O2 was added, the mixture was incubated in the dark and room temperature for 5 min. The 75 µL 1,10 phenanthrolines were added into the sample and control well and incubated again for 10 min in the dark and room temperature. Sample absorbance was measured at 510 nm (Prahastuti et al., 2019; Prahastuti et al., 2020; Girsang et al., 2020).

Percentage of H2O2 scavenging activity calculated with the equation:

$$\%\ H_2O_2\ \text{Scavenging Activity} = \frac{Sample\ Absorbance}{Control\ Absorbance} x\ 100 \quad (5)$$

2.6 DPPH Scavenging Activity

Briefly, 200 µl DPPH 0.077 mmol in methanol was added into 50 µl BTE in a microplate. Mixture incubated at room temperature for 30 minutes then absorbance measured at 517 nm with a microplate reader. Amount 250 µl DPPH used as negative control and 250 µl absolute DMSO used as blank (Prahastuti et al., 2019; Girsang et al., 2020; Prahastuti et al., 2020; Mawarni et al., 2020). Anti-oxidant activity of the DPPH method (%):

$$\%\ \text{DPPH Scavenging Activity} = \frac{Control\ \ Aborbance - Sample\ \ Absorbance}{Control\ Ab\ sorbance} x100 \quad (6)$$

2.7 ABTS Scavenging Activity

Briefly, 2 µl sample added into 96-well plate and then 198 µl ABTS working reagent added into the

sample well. 200 µl DMSO used as blank and 200 µl ABTS working reagent used as control. Plate incubated for 6 minutes at 37oC. The absorbance of the sample was measured at 745 nm (Prahastuti et al., 2019; Prahastuti et al., 2020; Girsang et al., 2020; Mawarni et al., 2020). Anti-oxidant activity of the ABTS method (%):

$$\% \text{ ABTS Reducing Activity} = \frac{Control\ Ab - Sample\ Ab}{Control\ Ab} x\ 100 \quad (7)$$

2.8 Collagenase Inhibition Activity

Method to measure the inhibition activity of collagenase based on the method by Sigma Aldrich with a little modification (Widowati et al., 2018a; Utami et al., 2018 Girsang et al., 2020). Sample mixture consist of 30 µl sample (7.81-250 µg/ml), 10 µl Collagenase from Clostridium histolyticum (0.1 mg/ml, Sigma C8051), and 60 µl tricine buffer (50 mM Tricine, 10 mM calcium chloride, 400 mM sodium chloride, pH 7.5) incubated at 37oC for 20 minutes. 10 µl enzyme and 90 µl phosphate buffer used as control and 10 µl enzyme, 80 µl phosphate buffer, and 30 µlsample used as blank. 20 µl FALGPA (1 mM, Sigma F5135) was added to each well except blank. Sample absorbance was measured at 335 nm. Inhibition activity calculated with the equation:

$$\% \text{ Collagenase Inhibition} = \frac{Control\ \ Absorbance - Sample\ \ Absorbance}{Control\ Absorbance} x100 \quad (8)$$

2.9 Elastase Inhibition Activity

Method to measure the inhibition activity of elastase based on the method by Sigma Aldrich and Widowati et al. (2018a) with a little modification (Utami et al., 2018; Girsang et al., 2020; Mawarni et al., 2020). Sample mixture consist of 10 µl sample (2.08-66.67 µg/ml), 5 µl elastase from porcine pancreas (0.01 mg/ml, Sigma 45124) and 125 µl Tris buffer (100 mM, pH 8, Pharmacia Biotech 17-1321-01) incubated at 25oC for 15 minutes. Briefly 5 µl enzyme and 135 µl Tris buffer used as control and 130 µl Tris buffer and 10 µl sample used as blank. Amount 10 µl SucAla3-pNA was added to each well and incubated at 25oC for 15 minutes. Sample absorbance was measured at 410 nm. Inhibition activity calculated with the equation:

$$\% \text{ Elastase Inhibition} = \frac{Control\ \ Absorbance - Sample\ \ Absorbance}{Control\ Absorbance} x100 \quad (9)$$

2.10 Statistical Analysis

All data from H2O2, DPPH, and ABTS scavenging, collagenase, and elastase inhibition were analyzed statistically with ANOVA and Tukey HSD Post Hoc Test ($P<0.05$). Inhibitory Concentration (IC50) using linear regression analysis. The column graph was formed with Graph Pad 7 Prism.

3 RESULT AND DISCUSSION

3.1 Phytochemical Analysis

Phytochemical analysis was done to know what kind and how many secondary metabolite properties were from the sample. Even the data obtained as qualitative data, but data can become the base to pick the method to measure the total content of the secondary metabolite of the sample. BTE contains low content of alkaloid (+), not detected saponin (-), very high content of phenol (++++), low tannin (+), not detected steroid (-), high triterpenoid (+++), high terpenoid (+++), and moderate alkaloid (++).

This result is slightly different from the research done by Widowati et al. (2015) that showed black tea extract contains medium content on flavonoid, terpenoid, and phenol, low content on terpenoid, tannin, and saponin, and negative content on alkaloid and steroid. During fermentation, catechin is oxidized and polymerized into theaflavins (TF) and thearubigins (TR) or degraded to another form (Das and Datta, 2019). The difference of secondary metabolites of BTE is caused by different environmental and management factors (Ahmed et al., 2019) including plant genotype (Cherotich et al., 2013; Chen et al., 2018; Mu et al., 2018), shade (Sano et al., 2018), elevation (Han et al., 2017; Kfoury et al., 2018), drought (Scott et al., 2019), precipitation (Ahmed et al., 2014; Kowalsick et al., 2014), temperature (Ahmed et al., 2019), model of agricultural production (Ahmed et al., 2013; Han et al., 2018), microbes (Ahmed et al., 2019), and numerous pest insects (Scott and Orians, 2018). Because the source of used BTE in this research was different from previous research resulted different secondary metabolites (Ahmed et al., 2019). The different extraction solvents resulted in different compound content (Yusnawan, 2013). The different

extraction solvents resulted from different bioactivity (Ngo et al., 2017).

3.2 Total Phenolic and Flavonoid Content

Total phenol activity was used to know how many phenol contained in the sample by measure the sample + reagent with spectrophotometry. The phenol content of BTE is 52.81±1.38 µg QE/mg sample. Total flavonoid activity was used to know how many flavonoids were contained in the sample by measure the sample + reagent with spectrophotometry. The flavonoid content of BTE is 10.96±12.46 µg QE/mg sample.

Phenol total activity of BTE is 52.81 µg GAE/mg extract indicated that flavonoid is the most secondary metabolites in black tea. But the flavonoid total activity is 11.73 µg QE/mg extract, this result was in line with previous research that BTE has phenol standard catechin (14.33 µg Catechin/mg), kaempferol (4.33 µg kaempferol/mg), myricetin (4.17 µg myricetin/mg) dan quercetin (4.30 µg quercetin/mg) (Widowati et al., 2015).

3.3 H2O2 Scavenging Activity

BTE has anti-oxidant activity in scavenging H2O2 with a higher concentration of BTE increased the H2O2 scavenging activity (Figure 1).

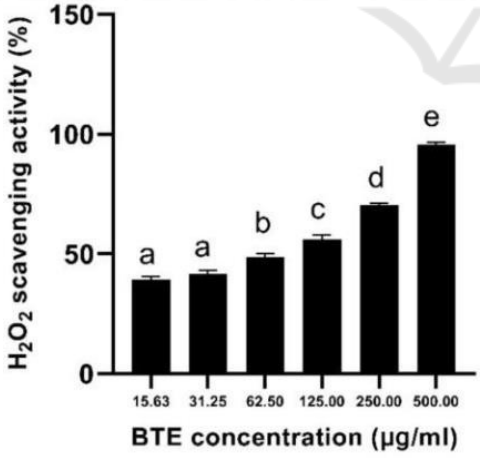

Figure 1: Anti-oxidant activity of BTE at various concentrations against H2O2 scavenging activity.

*The data was presented as mean ± SD. The assay was done in triplicate for each concentration. The different letter (a.b.c.d.e) showed significant difference among BTE concentration (P<0.05).

The IC50 value of H2O2 scavenging activity was 88.18 µg/ml, according to Marjoni and Zulfisa (2017), BTE was categorized as active when the IC50 value <100 µg/ml. The research was done by Fernando and Soysa (2015) the BTE has Effective Concentration 50 (EC50) at 91.96 µg/ml. The EC50 means the half maximal effective concentration extract to scavenge the H2O2 that same with IC50. The IC50 of BTE is nearly the same that means the result is valid.

3.4 DPPH Scavenging Activity

BTE has anti-oxidant activity in scavenging DPPH with higher concentration BTE increased the DPPH scavenging activity (Figure 2). The IC50 value of DPPH scavenging activity of BTE is 15.29 µg/ml which means with 15.29 µg/ml extract can inhibit 50% of DPPH become lost its radical properties, it was categorized highly active anti-oxidant (Marjoni and Zulfisa, 2017).

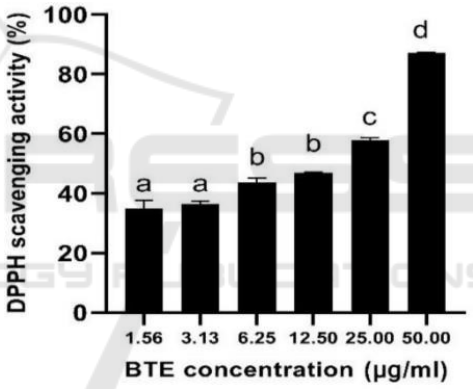

Figure 2: Anti-oxidant activity of BTE at various concentrations against DPPH scavenging activity.

*The data was presented as mean ± SD. The assay was done in triplicate for each concentration. The different letter (a.b.c.d) showed significant difference among BTE concentration (P<0.05).

Research by Leslie & Gunawan (2019), the DPPH scavenging activity of BTE has IC50 137.60 µg/ml. The difference in the value of IC50 means that the BTE has the differences between each research. The differences included the genotype, the method of extraction, the source of black tea, etc. The DPPH scavenging activity of BTE was lower than previous research (0.48 µg/ml) because, in this research using filler mannitol, lactose, and starch 10-50% which added in extract, it will lower the active compound of BTE and decrease the anti-oxidant activity.

3.5 ABTS Reducing Activity

BTE has anti-oxidant activity in reducing ABTS with higher concentration BTE increase the ABTS reducing activity (Figure 3). The IC50 value ABTS reducing the activity of BTE was 17.21 µg/ml, it was categorized highly active anti-oxidant (Marjoni and Zulfisa, 2017).

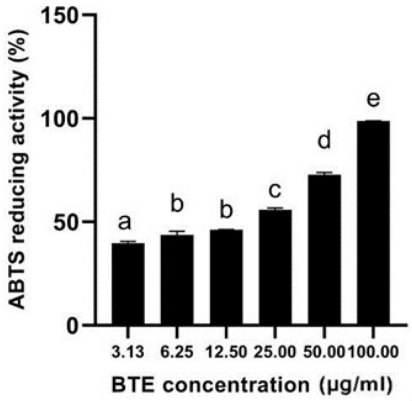

Figure 3: Anti-oxidant activity of BTE at various concentrations against ABTS scavenging activity.

*The data was presented as mean ± SD. The assay was done in triplicate for each concentration. The different letter (a.b.c.d.e) showed significant difference among BTE concentration (P<0.05).

The IC50 value of ABTS scavenging activity of BTE was 17.68 µg/ml which means with 15.29 µg/ml, it was categorized as a very strong activity (Marjoni and Zulfisa, 2017). This result was similar to previous research that BTE had active anti-oxidant because it contained high secondary metabolites namely epigallocatechin gallate (EGCG)> epigallocatechin (EGC)>epicatechin (EC) = catechin. EGCG is the most effective anti-oxidant polyphenol against free radicals (He et al., 2018). *C. sinensis* tea contains high polyphenol (30%) with EGCG consists of 9% of that total polyphenol (Crozier et al., 2012).

3.6 Skin Anti-aging Activity

The result of the skin anti-aging activity is shown in Figure 4 and Table 1. Based on the data (Figure 4, Table 1) showed that a higher concentration of BTE increased inhibition activity toward elastase and collagenase inhibition. Based on the result, the IC50 value of collagenase inhibition activity of BTE was categorized as less active in skin anti-aging activity when the IC50 value > 100.00 µg/ml (Vijayakumar et al., 2017)., and BTE has categorized moderate active elastase inhibition activity (IC50: 15.01-50.00 µg/ml) (Vijayakumar et al., 2017).

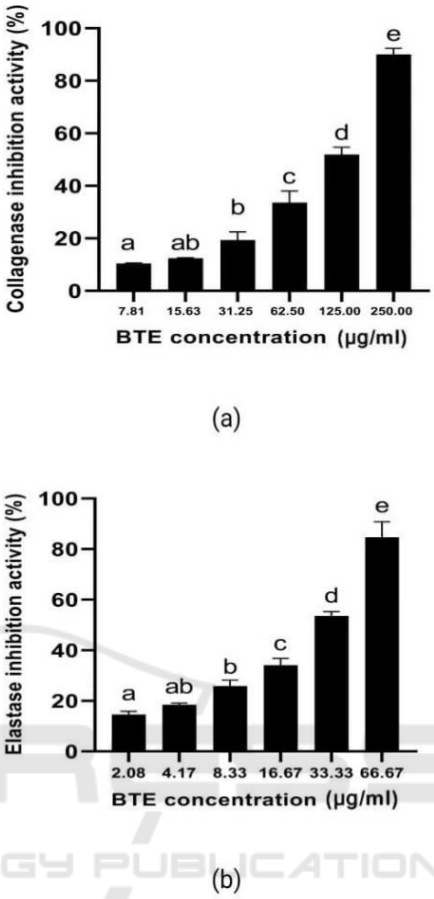

Figure 4: Skin anti-aging activity of BTE at various concentrations against collagenase and elastase inhibition activity.

*The data was presented as mean ± SD. The assay was done in triplicate for each concentration. The different letter (a.ab,bc.d.e) for collagenase and elastase inhibition showed significant difference among BTE concentration (P<0.05).

Table 1: The value of IC50 for collagenase and elastase inhibition activity of BTE.

Sample	IC_{50} of Collagenase Inhibition Activity (µg/ml)	IC_{50} of Elastase Inhibition Activity (µg/ml)
BTE	123.72 ± 2.44	31.34 ± 1.70

*The data was presented as mean ±SD. The IC_{50} value was calculated according to regression linear y=a+bx

BTE has collagenase and elastase inhibition activity with IC50 values is 123.72 µg/ml and 31.34 µg/ml, respectively. From the IC50 value, BTE has

higher elastase inhibition activity than collagenase. The anti-aging properties of black tea are because of the presence of high polyphenol content. The polyphenol that is responsible for anti-aging properties is catechin (Khanna & Maurya, 2012). The hydroxyl groups of polyphenol and flavonoid chemicals are effective at forming bonds with the carboxyl groups of the serine amino acid at the elastase enzyme's active site, altering the enzyme's mechanism of action. As a result, elastase is unable to cleave peptide bonds, which considerably aids in the prevention of skin elasticity loss and wrinkle formation (Vijayakumar et al., 2017).

Collagenase is a zinc-containing metalloproteinase, catechins are known as metal chelators that may bind to the Zn2+ ion within collagenase that prevent it from binding with the substrate (Zeng et al., 2019; Voos et al., 2021). Therefore, polyphenol content may bind to the Zn ionactive site, preventing the substrate from digesting the enzyme, and this mechanism could contribute to the collagenase inhibition activity of BTE (Pientaweeratch et al., 2016).

4 CONCLUSIONS

Black tea extract contains phenolic compound 52.81µg QE /mg sample and total flavonoid 10.96 µg QE /mg sample. The IC50 of black tea extract in H2O2 scavenging activity 88.17±1.69 µg/ml, DPPH scavenging activity 15.29±0.31 µg/ml and ABTS 17.21±1.22 µg/ml. Black tea extract has active to highly active anti-oxidant properties. Anti-aging activity namely collagenase inhibition with IC50 123.72±2.44 µg/ml as less activity and elastase inhibition with IC50 31.34±1.70 µg/ml as moderate active activity show that black tea extract has less and moderately active in anti-aging properties.

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REFERENCES

Adnyana, I. K., Abuzaid, A. S., Iskandar, E. Y. and Kurniati, N. F. (2016). 'Pancreatic lipase and α-amylase inhibitory potential of mangosteen (Garcinia mangostana Linn.) pericarp extract'. Int. J. Med. Res. Health Sci. 5(1), 23- 28.

Ahmed, S., Griffin, T.S., Kraner, D., Schaffner, M.K., Sharma, D., Hazel, M., Leitch, A.R., Orians, C.M., Han, W., Stepp, J.R. and Robbat, A. (2019). 'Environmental factors variably impact tea secondary metabolites in the context of climate change'. Front Plant Sci. 10(939), 1- 16.

Ahmed, S., Peters, C.M., Chunlin, L., Meyer, R., Unachukwu, U., Litt, A., Kennelly, E., Stepp, J.R. (2013). 'Biodiversity and phytochemical quality in indigenous and state-supported tea management systems of Yunnan, China'. Conserv. Lett. 6, 28–36.

Ahmed, S., Stepp, J.R., Orians, C., Griffin, T., Matyas, C., Robbat, A., Cash, S., Xue, D., Long, C., Unachukwu, U., Buckley, S. (2014). 'Effects of extreme climate events on tea (Camellia sinensis) functional quality validate indigenous farmer knowledge and sensory preferences in tropical China'. PLoS One. 9(10),e109126.

Asan, T., Lister, I.N.E., Fachrial, E., Widowati, W., Samin, B., Liena, L. (2019). 'Potency of black soybean (Glycine max (L.) Merr) extracted and daidzein as anti-oxidant and antihyaluronidase'. Trad. Med. J. 24(1), 52-58.

Bera, T. K., Chatterjee, K., Ghosh, D. B. G. M. (2015). 'In vitro anti-oxidant properties of the hydro-methanol extract of the seeds of Swienia mahagoni (L.) Jacq'. Biomark. Genom. Med. 7, 18-24.

Chen, S., Li, M., Zheng, G., Wang, T., Lin, J., Wang, S., Wang, X., Chao, Q., Cao, S., Yang, Z., Yu, X. (2018). 'Metabolite profiling of 14 wuyi rock tea cultivars using UPLC-QTOF MS and UPLC-QqQ MS combined with chemometrics'. Molecules. 23(2), 104-121.

Cherotich, L., Kamunya, S.M., Alakonya, A., Msomba, S.W., Uwimana, M.A., Wanyoko, J.K., Owuor, P.O. (2013). 'Variation in catechin composition of popularly cultivated tea clones in East Africa (Kenya)'. Am. J. Plant Sci. 4(3), 628-640.

Crozier, A., Clifford, M. N., & Del Rio, D. (2012). Bioavailability of dietary monomeric and polymeric flavan-3-ols. Bioavailability and function of flavonoids: oxidative stress and disease, 30, 45-78.

Das, S., Samanta, T., Datta, A.K. (2019). 'Analysis and modeling of major polyphenols during oxidation in production of black tea'. J. Food Process. Preserv. 43(12), e14283.

Eric R.S., Xin L., Ji-Peng W., Nicole K., Joshua M., Ming- Ming G., Amma A., Albert R.Jr., Selena A., Sean B.C., Timothy S.G., John R. S., Wen-Yan H., Colin M. O. (2020). 'Changes in tea plant secondary metabolite profiles as a function of leafhopper density and damage. Front. Plant. Sci. 11(636), 1-15.

Fernando, C.D., Soysa, P. (2015). 'Optimized enzymatic colorimetric assay for determination of hydrogen peroxide (H2O2) scavenging activity of plant extracts'. Methods X. 2(2015), 283-291.

Girsang, E., Lister, I.N.E., Ginting, C.N., Sholihah, I. A., Raif, M., A., Kurniadi, S., Million, H., Widowati, W. (2020). 'Anti-oxidant and skin anti-aging activity of rutin and caffeic acid.' Pharmaciana. 10(2), 147-156.

Girsang, E., Lister, I.N.E., Ginting, C.N., Khu, A., Samin, B., Widowati, W., Wibowo, S., Rizal, R. (2019). 'Chemical constituents of snake fruit (Salacca zalacca (Gaert.) Voss) peel and in silico anti-aging analysis'. Mol. Cell. Biomed. Sci. 3(2), 122-128.

Han, W.Y., Huang, J.G., Li, X., Li, Z.X., Ahammed, G.J., Yan, P.. Stepp, J.R. (2017). 'Altitudinal effects on the quality of green tea in east China: a climate change perspective'. Eur. Food Res. Technol. 243(2), 323–330

Han, W.-Y., Wang, D.-H., Fu, S.-W., Ahmed, S. (2018). 'Tea from organic production has higher functional quality characteristics compared with tea from conventional management systems in China.' Biol. Agric. Hortic. 34, 120–131

He J., Lei X., Le Y., Xiaofeng W. (2018). 'Epigallocatechin gallate is the most effective catechin against anti-oxidant stress via hydrogen peroxide and radical scavenging activity'. Med. Sci. Monit. 24, 8198–8206.

Holmbeck H., Birkedal-Hansen H. (2013). 'Collagenases. encyclopedia of biological chemistry'. Academic Press

Jusri, R., Widodo, W. S., Widowati, W., Sormin, D. E., Irmansyah, A., Fachrial, E., et al. (2019) 'Comparison of anti-oxidant and anti-hyaluronidase potentials of pineapple core extract (Ananas comosus (L.) Merr.) and luteolin.' MKB. 51(2), 63–69.

Kfoury, N., Morimoto, J., Kern, A., Scott, E. R., Orians, C. M., Ahmed, S., et al. (2018). 'Striking changes in tea metabolites due to elevational effects'. Food Chem. 264, 334–341.

Khanna, A., Maurya, P.K. (2012). 'Role of tea catechins in prevention of aging and age-related disorders'. CellMed. 2(1), 2-1.

Kowalsick, A., Kfoury, N., Robbat Jr, A., Ahmed, S., Orians, C., Griffin, T., Cash, S.B., Stepp, J.R. (2014). 'Metabolite profiling of Camellia sinensis by automated sequential, multidimensional gas chromatography/mass spectrometry reveals strong monsoon effects on tea constituents'. J. Chromatogr. A 1370, 230–239

Leslie, P.J., Gunawan, S. (2019). 'Uji fitokimia dan perbandingan efek antioksidan pada daun teh hijau, teh hitam, dan teh putih (Camellia sinensis) dengan metode DPPH (2,2-difenil-1- pikrilhidrazil)'. Tarumanagara Medical Journal 1(2).

Liana, L., Rizal, R., Widowati, W., Fioni, F., Akbar, K., Fachrial, E., Lister, I.N.E. (2019). 'Anti-oxidant and anti-hyaluronidase activities of dragon fruit peel extract and kaempferol-3-o-rutinoside'. J. Kedokt. Brawijaya. 30(4), 247-252.

Mancini M., Anna M.L., Gaelle S., Christian M., Nicola D.D., Gerry M., and Eleonora C. (2014). 'MicroRNAs in human skin ageing'. Ageing Res. Rev. 17, 9-15.

Marjoni, M.R. Zulfisa, A. (2017). 'Anti-oxidant activity of methanol extract/fractions of Senggani leaves (Melastoma candidum D. Don)'. Pharm. Analytic. Acta. 8(8):1-6.

Mawarni, E., Ginting, C. N., Chiuman, L., Girsang, E., Handayani, R. A. S., Widowati, W. (2020). 'Anti-oxidant and elastase inhibitor potential of petals and receptacle of rose flower (Rosa damascena)'. Pharm. Sci. Res. 7(2), 105-113.

Mu, B., Zhu, Y., Lv, H.-P., Yan, H., Peng, Q.-H., Lin, Z. (2018). 'The enantiomeric distributions of volatile constituents in different tea cultivars'. Food Chem. 265, 329–336.

Mukhopadhyay, D., Dasgupta, P., Roy, D. S., Palchoudhuri, S., Chatterjee, I., Ali, S., Dastidar, S. G. (2016). 'A Sensitive in vitro spectrophotometric hydrogen peroxide scavenging assay using 1, 10-phenanthroline.' Free Radicals & Anti-oxidants. 6(1), 124-132.

Ngo, T.V., Scarlett, C.J., Bowyer, M.C., Ngo, P.D., Vuong, Q.V. (2017) 'Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of Salacia chinensis L'. J. Food Quality. ID 9305047 :1-8

Pientaweeratch, S., Panapisal, V., Tansirikongkol, A. (2016). 'Antioxidant, anti-collagenase and anti-elastase activities of Phyllanthus emblica, Manilkara zapota and silymarin: An in vitro comparative study for anti- aging applications'. Pharm. Biol. 54(9), 1865-1872.

Prahastuti, S., Hidayat, M., Hasianna, S.T., Widowati, W., Amalia, A., Yusepany, D.T., Rizal, R., and Kusuma, H.S.W. (2019). 'Anti-oxidant potential ethanolic extract of Glycine max (l.) Merr. Var. Detam and daidzein.' J. Physics: Conf. Series. 1374, 1-12.

Prahastuti, S., Hidayat, M., Hasiana, S.T., Widowati, W., Widodo, W.S., Handayani, A.S., Rizal, R., Kusuma, H. S. W. (2020). 'The ethanol extract of the bastard cedar (Guazuma ulmifolia L.) as anti-oxidants'. Pharmaciana. 10(1), 77-88.

Sano, T., Horie, H., Matsunaga, A., and Hirono, Y. (2018). 'Effect of shading intensity on morphological and color traits and on chemical components of new tea (Camellia sinensis L.) shoots under direct covering cultivation.' J. Sci. Food Agric. 98, 5666–5676.

Scott, E. R., Li, X., Kfoury, N., Morimoto, J., Han, W.-Y., Ahmed, S., et al. (2019). 'Interactive effects of drought severity and simulated herbivory on tea (Camellia sinensis) volatile and non-volatile metabolites.' Environ. Exp. Bot. 157, 283–292

Scott, E. R., and Orians, C. M. (2018). 'Differential changes in tea quality as influenced by insect herbivory.' in Stress physiology of tea in the face of climate change, eds W.-

Y. Han, X. Li, G. J. Ahammed (Singapore: Springer), 217–240 Siregar, I.D., Kusuma, H.S.W., Widowati, W., Marpaung, H.H., Ferdinand, S., Fachrial, E.,

Lister, I.N.E., 2019. 'Anti-oxidant and antityrosinase activities of ethanolic Pachyrhizus erosus peel and tuber extract'. MKB. 51(2), 75-81.

Sudaryat, Y., Kusmiyati, M., Pelangi, C. R., Rustamsyah, A. 2015. 'Aktivitas antioksidan seduhan sepuluh jenis mutu teh hitam (Camellia sinensis (L.) O. Kuntze) Indonesia Anti-oxidant activity of ten grades of Indonesia black tea'. Jurnal Penelitian Teh dan Kina. 18(2), 95–100.

Utami S, Sachrowardi QR, Damayanti NA, Wardhana A, Syarif I, Nafik, S Arrahmani BC, Kusuma HSW, Widowati W. (2018). 'Anti-oxidants, anticollagenase and antielastase potentials of ethanolic extract of ripe sesoot (Garcinia picrorrhiza Miq.) fruit as skin anti-aging'. J. Herbmed. Pharmacol. 7(2), 88-93.

Vijayakumar, R. A. M. Y. A., Gani, S., Mokhtar, N. (2017). 'Anti-elastase, anti-collagenase and antimicrobial activities of the underutilized red pitaya peel: An in vitro study for anti-aging applications'. Asian J. Pharm. Clin. Res. 10(8), 251-255.

Voos, K., Schönauer, E., Alhayek, A., Haupenthal, J., Andreas, A., Müller, R., Hartmann, R.W., Brandstetter, H., Hirsch, A.K.H., Ducho, C. (2021). 'Phosphonate as a stable zinc-binding group for "pathoblocker" inhibitors of clostridial collagenase H (ColH)'. ChemMedChem. 16(8), 1257-1267.

Vrianty, D., Qodariah, R.L., Widowati, W., Sinaga, A.P.F., Fibrina, D., Fachrial, E., Lister, I.N.E. (2019). 'Comparison of anti-oxidant and anti-tyrosinase activities of pineapple (Ananas comosus) core extract and luteolin compound'. J. Kedok. Brawijaya. 30(4), 240-246.

Widowati, W., Herlina, T., Ratnawati, H., Constantia, G., Deva, I.D.G S, Maesaroh, M. (2015). 'Anti-oxidant potential of black, green and oolong tea methanol extracts.' Biol. Med. Nat. Prod. Chem.4(2), 38-43.

Widowati, W., Fauziah, N., Herdiman, H., Afni, M., Afifah, E., Kusuma, H. S. W., Nufus, H., Arumwardana, S,Rihibiha, D.D. (2016). 'Anti-oxidant and anti-aging assays of Oryza sativa extracts, vanillin and coumaric acid'. J. Nat. Remed. 16(3), 88-99.

Widowati, W., Rani, A. P., Hamzah, R. A., Arumwardana, S., Afifah, E., Kusuma, H. S. W., Rihibiha, D. D., Nufus, H., Amalia, A. (2017). 'Anti-oxidant and skin anti-aging assays of Hibiscus sabdariffa extract and its compounds'. Nat. Prod. Sci. 23(3), 192-200

Widowati, W., Janeva, W. B., Nadya, S., Amalia, A., Arumwardana, S., Kusuma, H. S. W., Arinta, Y. (2018a). 'Anti-oxidant and skin anti-aging activities of Jasminum sambac extract, and its compounds.' J. Rep. Pharm. Sci. 7(3), 270-285

Widowati W, Noverina R, Ayuningtyas W. Kurniawan D, Faried A, Laksmitawati DR, Rihibiha DD, Rizal R, Suciati T, Sumitro SB. Recative oxygen species and aging mechanism (2018b). In Wilkerson S ed., Reactive oxygen species (ROS) mechanism and role in health and disease. Nova Science Publishers, New York, p 101-134 Wittenauer, J., Mackle, S., Submann, D., Schweiggert- Weisz, U., Carle, R. (2015). 'Inhibitory effects of polyphenols from grape pomace extract on collagenase and elastase activity'. Fitoterapia. 101, 179 – 187.

Yadav, A., Rewa, K., Ashwani, Y., Mishra, J.P., Seweta, S., Shashi, P. (2016). 'Anti-oxidants and its functions in human body - a review'. Res. Environ. Life Sci. 9(11), 1328-1331.

Yusnawan, E. (2013). 'The effectiveness of polar and non-polar fractions of Ageratum conyzoides L. to control peanut rust disease and phytochemical screenings of secondary metabolites'. J. HPT Tropika, 13(2):159-166.

Zeng, X., Du, Z., Xu, Y., Sheng, Z., Jiang, W. (2019). 'Characterization of the interactions between apple condensed tannins and biologically important metal ions [Fe2+ (3d6), Cu2+ (3d9) and Zn2+ (3d10)].' LWT. 114(2019), 108384.

Zhang S., Duan E. (2018). 'Fighting against skin aging the way from bench to bedside. Cell Transplant. 27(5), 729– 738.