Antioxidant Activity Test of Green Tea (*Camellia sinensis* L. Kuntze) Ethanolic Extract using DPPH Method

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Abstract: Green tea (*Camellia sinensis* (L.) Kuntze) is believed to have efficacy as a powerful antioxidant since it contains polyphenols that can reduce free radicals. The aim of this study was to determine the class of chemical compounds of the green tea leaf powder, to characterize the green tea ethanolic extract and to evaluate the antioxidant activity of the green tea ethanolic extract with DPPH method. Green tea was extracted by maceration method using 96% of ethanol and followed by the phytochemical screening, characterization and the antioxidant activity test by DPPH method. The ethanol extract of green tea contained flavonoids, saponins, tannins and steroids / triterpenoids. The moisture content of green tea extract was 14.25%; the total ash was 1.06% and the acid insoluble ash content was 0.099%.The antioxidant activity assay gave 11.83 ± 0.005 µg/ml of IC_{50}. It can be concluded that the ethanol extract of green tea has a very strong antioxidant activity.

1 INTRODUCTION

Antioxidant is a compound that can counteract or mitigate the negative effects of free radicals in the body by donating an electron to the free radical so that its activity is inhibited (Ramadan, 2015). Antioxidants are needed to overcome the presence of free radicals, whereby they are not only addressed for health, but also to take care and beautify the skin including preventing the premature aging (Anggai et al., 2015). Tea (*Camellia sinensis*) is one of the plants that popular as a drink (Rohdiana, 2009). These plants contain flavonoids, the potent antioxidant derived from polyphenol compounds (Sudrajat et al., 2015). There are a wide variety of teas known in the community, including green tea. According to Dewi et al, green tea has a powerful antioxidant activity because the process of making green tea is not fermented like the other types of tea (Dewi et al., 2009). Based on the described facts, the researchers were interest to do the phytochemical screening, characterization and evaluation of the antioxidant activity of the ethanol extract of green tea (*Camellia sinensis* (L.) Kuntze).

2 METHODS

2.1 Phytochemical Screening

The phytochemical screening was done to determine the class of chemical compounds contained in the green tea leaves dried powder, including groups of alkaloids, flavonoids, saponins, tannins, and steroids / triterpenoids (Ministry of Health Republic of Indonesia, 1995).

2.2 Preparation of Green Tea Ethanolic Extract (GTEE)

Green tea leaves powder was extracted by maceration method using 96% of ethanol. The macerate was then evaporated with a rotary evaporator at a temperature of 40-50 ^{0}C to obtain a viscous extract (Directorate General 0f Drugs and Food Control, 1979).

The yield value was calculated by comparing the obtained extract with the original amount of green tea powder and stated as percentage.

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2.3 GTEE Characterization

The characterization of the GTEE included the determination of moisture content using azeotropic distillation method, the determination of total ash according to SNI 01-2891-1992, gravimetric assay and acid insoluble ash based on Indonesian Materia Medica (Ministry of Health Republic of Indonesia, 1995; Indonesian National Standard, 1992).

2.4 Antioxidant Activity Test

Antioxidant activity test of GTEE was evaluated by using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) Free Radical Scavenger method as described in Molenux with slight modification (Molyneux, 2004). Measurement of antioxidant activity was performed using UV-Vis spectrophotometer. Maximum wavelength results obtained were 516 nm. The measurement of the antioxidant activity used quercetine as the positive control with several concentration levels which was then defined in the quercetine calibration curve with regression equation $Y = 3.9734X + 3.0115$.

3 RESULTS AND DISCUSSION

3.1 Phytochemical Screening Results

The results of phytochemical screening of green tea powder can be seen in Table 1.

Table 1: Phytochemical screening test results of GTEE.

Compound classification	Result
Alkaloids	-
Flavonoids	+
Saponin	+
Tannin	+
Steroids / Triterpenoids	+

(+) Positive: containing the compound
(-) Negative: do not contain the compound

The dried green tea leaves powder are extracted by maceration which is a cold extraction method that processes several times extraction with shaking or stirring at room temperature, so that the substances contained in the sample are relatively stable compared to the heat extraction. The solvent used in the extraction process was 96% of ethanol which is relatively safe compared to the methanol and has a nature that may extract all of the secondary metabolites in botanical sample.

3.2 Characterization of GTEE

Approximately 500 grams of green tea powder gave extracts as much as 97.10 g (19.42%). This result fulfilled the Indonesian Herbal Pharmacopoeia which stated that the yield requirements for the ethanol extract of green tea is not less than 7.8%. The GTEE can be seen in the Figure 1.

Figure 1: Green tea ethanolic extract (GTEE) .

Characteristics of the GTEE were dark green colour, has bitter taste and specific odor. The results of determination of moisture content, total ash and acid insoluble ash content in GTEE were shown in Table 2.

Table 2: The characteristics of GTEE.

Parameters	Results (%) (n=3)
Water content	14.25 ± 0.46
Ash content	1.06 ± 0.13
Acid insuble ash content	0.099 ± 0.013

The determination of water content was performed in the GTEE since the water will allow the growth of microbes and affect the quality of GTEE during storage. The results of water content in GTEE amounted to 14.25 ± 0.46%. Based on the water content value, GTEE was included in the group of condensed extract which the water content is less than 16%.

The determination of total ash content was made to provide an overview mineral content contained in the green tea leaves powder as well as the contamination during the process of making the extract. The result of the determination of total ash content of GTEE fulfilled the requirements according to Indonesian National Standardization (SNI 01-2891-1992) that stated the maximum of total ash content is 8% (Indonesian National Standard, 1992).

3.3 Antioxidant Activity

One of the most common methods used to test the antioxidant activity is the method using a radical scavenger, 1,1-diphenyl-2- picrylhydrazil (DPPH). Measurement of radical scavenger is a method of measuring antioxidant that is simple, fast and does not require a lot of reagents compared to other methods that require considerable chemical reagents, analysis time and expensive. In this method, DPPH solution acts as a free radical that will react with antioxidant compounds. It will bind the electrons of the antioxidants as the test material which is characterized by changing the purple colour into pink or pale yellow (Molyneux, 2004).

Measurement of antioxidant activity by radical scavenger method using DPPH in dark purple methanol was detected at visible wavelengths of about 500-520 nm. The parameter to interpret the results of DPPH testing was with IC_{50} (Inhibitor Concentration) values. IC_{50} is the concentration of the substrate or sample solution that can reduce DPPH activity by 50%. The smaller the IC_{50} value means the higher the antioxidant activity (Prakash, 2001).

IC_{50} values were obtained based on the calculation of linear regression equation obtained by plotting concentration of the test solution and DPPH damping percentage as parameter of antioxidant activity, where the concentration of test solution (µg/ml) as X axis and the percentage of damping as Y axis. IC_{50} of GTEE and control values can be seen in Table 3.

Table 3: Antioxidant activity (IC_{50}) GTEE values compared to controls.

Sample	IC_{50} (µg/ml)
Green Tea Ethanolic Extract (GTEE)	11.83 ± 0.005
.Quercetine	2.32 ± 0.006

According to Mardawati, et al. (2008) GTEE antioxidant activity can be categorized as very strong antioxidant. Green tea ethanolic extract has high antioxidant activity because it contains flavonoids compound which contains hydroxyl group that donates hydrogen to the free radical. The compound can neutralize the free radical by giving electrons, therefore atom with the unpaired electron can get electron and no longer become free radical.

4 CONCLUSION

Green tea ethanolic extract contains flavonoids, saponins, tannins and steroids / triterpenoids compounds. It has moisture content of 14.25 ±0.46%, total ash of 1.06 ± 0.13% and acid insoluble ash content of 0.099 ± 0.013%, and shows very strong antioxidant activity.

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