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Potency of Industrial Tea Waste: Comparison Between Green And Black Tea Industrial Wastes as UV Filter for Sunscreen

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Abstract

Indonesia is one of ten biggest countries that produce tea for world. It makes Indonesia produce various tea products. Every tea production process produces the large quantity of industrial tea wastes every day. Our previous research showed that industrial tea wastes still have antioxidant activity. It means that industrial tea wastes contained of phenolic compounds which can be used as UV filter for sunscreen. This research compared antioxidant activity, total phenolic contents and UV filter effectiveness between green and black industrial tea wastes. Antioxidant activity were analyzed by reducing power and DPPH method, total phenolic contents of tea wastes extract were analyzed using Folin-Ciocalteu assay, while UV filter effectiveness were assessed by UV spectra and absorbance of each tea wastes extract related to its concentration in order to yield maximum protection. The results showed that although green tea waste extract had higher antioxidant activity but adversely, black tea had higher total phenolic contents. UV filter effectiveness is affected by polyphenols content in substances, so it suggested that black tea waste extract is more potential than green tea waste extract as photoprotection substance.

Keywords: tea waste, UV filter, sunscreen

INTRODUCTION

Solar UV light reaching earth is a combination of both UVB (290–320 nm) and UVA (320–400 nm) wavelengths. In the past two decades, a dramatic increase has been noted in the incidence of non melanoma skin cancer (NMSC). The major environmental influence on the development of NMSC is exposure to ultraviolet (UV) radiation, which generates reactive oxygen species and free radicals that damage a variety of cellular molecules. In animal models of chemical and ultraviolet-induced carcinogenesis, green and black teas demonstrate anti-tumor activity even when administered after UVB exposure (Rees et all., 2007).

Green and black teas are derived from the same plant, *Camellia sinensis*, after the leaves are "fermented" for different lengths of time. Both contain polyphenols, antioxidants that scavenge reactive oxygen and nitrogen species, and bind proteins (Zhen, 2002). Tea catechins are a group of important protective sunscreen compounds (Mamati et al., 2004).

Indonesia is one of ten biggest countries that export tea. It means that Indonesia has many tea industries (Anonym, 2008). Every tea industry produces tea product and tea waste. There are abundant industrial tea wastes, both of green and black tea. Our previous study showed that both of green and black tea wastes extract still have antioxidant activity and phenolic compounds. Because of phenolic compounds capable to scavenge free radicals from UV exposure, we interest to investigate antioxidant activity, total phenolics and UV filter effectiveness of both green and black industrial teas waste.

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METHODS

Chemicals.

Samples, both of green and black tea wastes was obtained from beverages company in Bawen, Semarang, Central Java. DPPH and Folin-Chiocalteou phenol reagent, tri-chloro acetic (TCA), $FeCl_3$, and $K_4Fe(CN)_6$ were obtained from Merck. All solvent such as methanol and ethyl acetic were also purchased from Merck, Germany.

Tea Waste Extract Preparation

100 gram of each tea waste (green and black tea) was extracted with methanol 80% (graded, 4 x 500 mL). Solution was filtered and filtrate was vacuumed by rotary evaporator until volume decrease three-fourth part. Filtrate gained was extracted again with ethyl acetic (graded, 1 x 150 mL followed by 4 x 50 mL). Filtrate was concentrated again by rotary evaporator. Concentrated extract was dried by nitrogen gas and kept in refrigerator at 5^oC.

Determination of Total Phenolic Content (Singleton et al., 1999)

The total phenolic contents of extracts was determined using to the Folin-Ciocalteu method. 1 mL extracts were added with 2 mL Folin-Ciocalteu reagent, and the reaction was neutralized with 2.5 mL sodium carbonate 7.5%. The absorbance of the resulting blue color was measured at 765 nm after 30 min. Using galic acid as standard total phenolic content was expresed as mg GA equivalent/gram of sample. Data reported of three replications.

Assay of Reducing Power (Koleva et al., 2007)

1 ml of extract solution was mixed with 2.5 ml phosphate buffer(0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [$K_3Fe(CN)_6$] 1%, then mixture was incubated at 50 degree C for 20 minutes. Two and one-half, 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ 0.1% and absorbance measured at 700nm in UV-Visible Spectriphotometer (Shimadzu, UVMini 1240). Potassium ferrocyanide was used as standard. Increased absorbance of the reaction mixture indicates stronger reducing power.

DPPH Radical Scavenging Activity (Lim et al., 2006)

The free radical scavenging capacity of tea waste extracts was determined using DPPH method. DPPH solution 0.02 mM was prepared in 95% methanol. Tea waste extract solution was made into several dissolutions. 1 mL of each dissolution was added with 2 mL DPPH 0.02 mM. Control solution was made from 1 mL of methanol 80% and was added by 2 mL DPPH 0.02 mM. As blank was 3 mL methanol 80%. Sample solution was then incubated for 30 min and absorbance of each solution was measured at 517 nm. Scavenging of the DPPH free radical was measured using the following equation:

$$\text{DPPH radical scavenging} = \frac{(A_{517}\ \text{control} - A_{517}\ \text{sample})}{A_{517}\ \text{control}} \times 100$$

Inhibition concentration of 50 % DPPH radical-scavenging sample was measured by linear regression method.

UV Filter Effectiveness Assay (Walter, 1997)

Optimum wavelength was determined by scanning sample solution in range 200 – 400 nm. Sample, both green and black tea waste extract was then dissolved with methanol 80% in various concentration, 60 µg/mL – 520 µg/mL. Sample solutions were then measured at optimum wavelength. UV filter effectiveness was determined by sample absorbance conversion following equation :

UV filter effectiveness = - log A

where A is absorbance of sample. UV filter effectiveness was categorized based on its value. Concentration optimal of extract that can be used as UV filter was determined by plotting maximum value in to formula from curve that related between UV protection factor vs. extract concentration.

DISCUSSION

Determination of total phenolic content

Folin–Ciocalteu is a method used for the determination of total phenolic compounds. The content of phenolic compounds is expressed as mg gallic acid equivalent (GAE) per gram sample. The amounts of total phenolics contents in industrial teas waste are shown in Table I. A higher content was observed in black tea waste in comparison with greean tea waste. Tea is known to have a high content of polyphenolics, about 36% polyphenols on a dry weight basis (Shahidi, 2000). As reported previously, theaflavin (TF) are low (2–6% of extracted solids) and thearubigens (>20%) are high in black tea, whereas in green tea, catechins are much higher (30–42%), particularly EGCG, which is the most abundant catechin (Lee and Lee, 2002).

Table I. Total Phenolic Contents (mg GAE/gram sampel) of Both Industrial Tea Wastes Green and Black Tea

Method	Industrial Tea Wastes	
	Green Tea	Black Tea
Folin-Ciocalteu	13.18 ± 1.15	35.77 ± 1.76

Evaluation of the antioxidant activity

Reducing Power Assay

Reducing power of both green and black teas waste were presented in Table 2.

The reducing ability of a compound generally depends on the presence of reductors which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom. The presence of reductors (i.e. antioxidants) in tea waste extracts causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm and was measured as mol equivalent $K_4Fe(CN)_6$/gram sample.

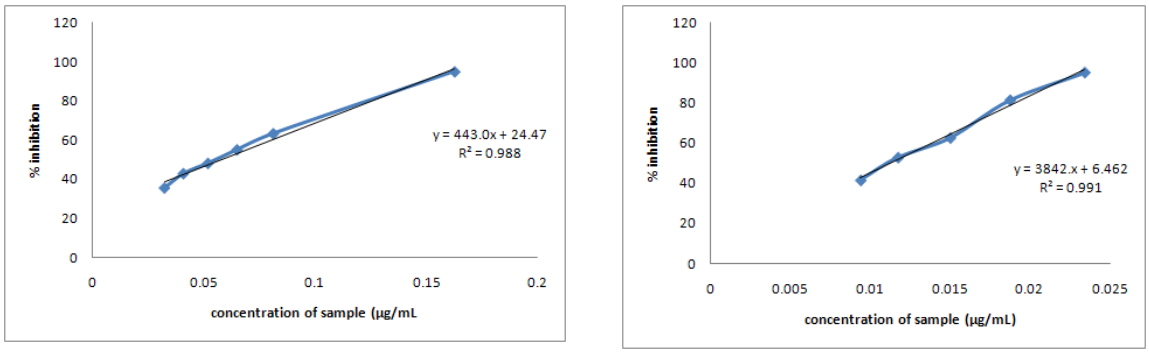
The result showed that green tea waste extract had higher antioxidant activity in comparison with black tea. This result was consistent with Lee and Lee (2002) that antioxidant activity of green tea extract was higher than black tea.

DPPH Assay

The concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely used to measure antioxidant activity. The lower the IC_{50} is the higher the antioxidant activity. The scavenging activity of both green and black teas waste extracts is shown in Table 2. Linear regression of both green and black industrial tea wastes to calculate IC_{50} were shown in Figure 1.

Table II. Antioxidant Activity of Both Green and Black Tea waste Extract (mek/gram sample)

Method	Industrial Tea Wastes	
	Green Tea	Black Tea
Reducing Power (mek/gram)	4.28 ± 0.21	2.38 ± 0.01
IC_{50} DPPH (µg/mL)	57.5	88.6



(a) (b)

Figure 1. Linear regression profile of concentration sampel vs % inhibition DPPH; (a) green tea waste extract; (b) black tea waste extract

Green tea waste extract had the higher hydrogen-donating capacity than black tea waste extract. This study has similarity with previous study (Lee and Lee, 2002) that the antioxidant capacity of green tea was much higher than black tea. Although black tea waste extract had higher total phenolic compounds than green tea waste extract, but green tea waste extract had higher antioxidant activity than black tea waste. The antioxidant capacity of tea is not related to a particular kind of polyphenol but to the combined activity of diverse antioxidants, including phenolic acids and polyphenols (Lee and Lee, 2002).

UV Filter effectiveness

UV Filter effectiveness of both green and black teas waste extract is shown in Table 3. Spectra scanned of both green and black tea waste extract showed that green tea waste extract had optimal absorption at 307 nm, but black tea waste extract had optimum absorption at 307 and 337 nm. This study showed that black tea waste had wider UV protection range than green tea. Black tea can absorb UVB and UVA light although in different intensity, while green tea waste only absorbs UVB light. This fact explained that polyphenols may also contribute to endogenous photoprotection. Spectra of each extract were shown in Figure 2.

Table III. UV Filter Effectiveness Concentration To Yield Maximum Protection

Method	Industrial Tea Wastes	
	Green Tea	Black Tea
Concentration of extract (µg/mL)	510	107.75

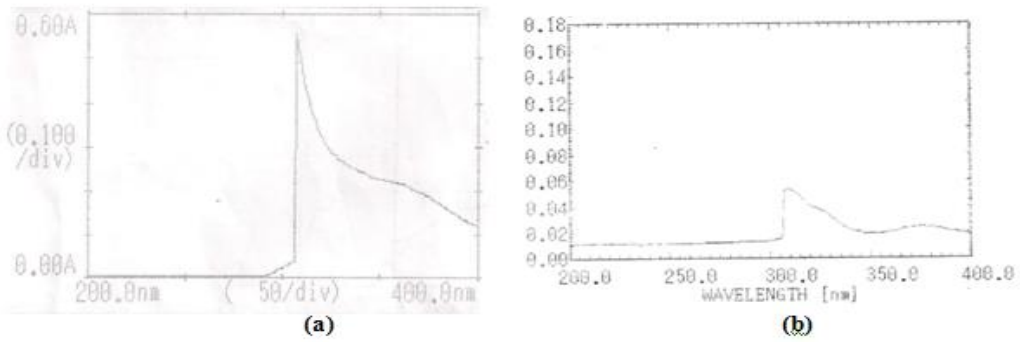


Figure 2. Industrial Tea Waste Extracts Spectra; (a) green tea; (b) black tea

This study also showed that black tea waste was more potent than green tea waste as UV filter because black tea waste had lower concentration (107.75 µg/mL) to yield maximum protection (15) than green tea waste (510 µg/mL). Figure 3. showed graph that related between UV protection factor vs. extract concentration. The result showed that photoprotection is affected by polyphenols contents in substances. Beside, structural requirement for suitable systemic photoprotection depent on the mechanism of action. One kind of the mechanism is increasing the barrier for UV light; e.g., UV-absorbing compounds (Sies and Stahl, 2004).

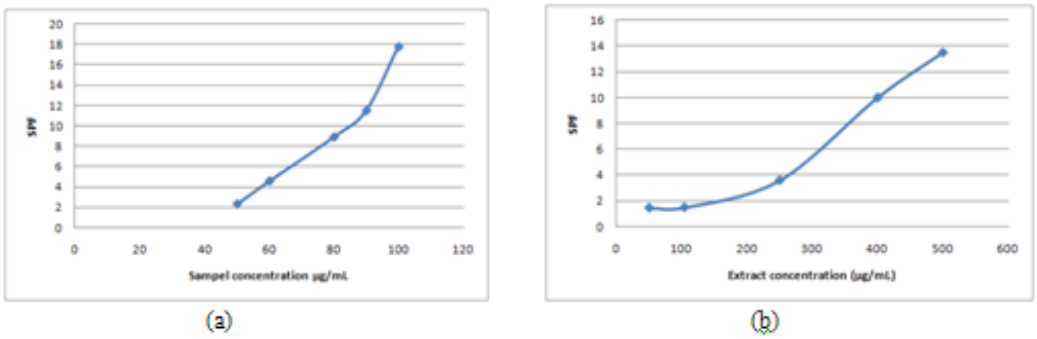


Figure 3. Graph of UV protector factor vs. extract concentration: (a) black tea waste; (b) green tea waste

CONCLUSION

Based on our study, we concluded that although green tea waste extract had higher antioxidant activity but adversely, black tea had higher total phenolic contents. UV filter effectiveness is affected by polyphenols content in substances, so it suggested that black tea waste extract is more potential than green tea waste extract as photoprotection substance.

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