Research Article

Antioxidant Activity and Determination of Total Polyphenol Levels in White Tea Leaves (*Camellia sinensis* L.)

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Abstract | White tea from the shoots is picked and dried by simple processing. Brewing white tea leaves have been shown to have antioxidant activity. This study aimed to determine the antioxidant activity of white tea leaves grown in Indonesia using extraction methods other than brewing, i.e., maceration. Study of free radical scavenging activity of 1,1- Diphenyl-2- Picrylhydrazyl (DPPH) and the determination of the total polyphenol content of white tea leaves have been conducted using UV-Visible spectrophotometry. Results of the study revealed that the antioxidant activities of the ethanol extract, n-hexane fraction, the water fraction and the ethyl acetate fraction of white tea leaves had IC_{50} values of 2.03, 4.77, 2.17 and 1.55 µg/mL, respectively. Quercetin was used for comparison which showed IC_{50} value of 1.05 µg/mL. The ethyl acetate fraction had an antioxidant activity, equivalent to quercetin, and can be categorized as a very strong antioxidant. The total polyphenol content of white tea leaves was 0.373±0.0064%. The result of thin-layer chromatography (TLC) profiles extract ethanol, n-hexane fraction and ethyl acetate fraction showed the presence of flavonoids, polyphenols, and monoterpene-sesquiterpenes. Ethyl acetate extract of white tea leaves has very strong antioxidant activity.

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Introduction

Free radicals in excess in the body are very dangerous because they cause damage to cells, nucleic acids, proteins, and fatty tissues. This cell damage can cause disease (Al-Nu'airat *et al.*, 2021). Reactive oxygen is a highly reactive oxygen-derived oxidizing compound consisting of free radicals and non-radical groups. Free radicals include superoxide anion (O_2), hydroxyl radical (OH), and peroxyl radicals (RO_2). Non-radical groups such as hydrogen peroxide (H_2O_2) and organic peroxides (ROOH) (Belambri *et al.*, 2018; Moghadam *et al.*, 2021; Li *et al.*, 2021). Reactive oxygen (reactive oxygen species or ROS) can be formed endogenously or exogenously as part of regular metabolic activity, physical activity, lifestyle, and diet (Bang *et al.*, 2021).

In our bodies, free radicals can be formed continuously, either through normal cell metabolic

processes, inflammation, malnutrition, or as a result of responses to external influences such as environmental pollution, ultraviolet (UV) light, cigarette smoke, and others (Al-Nu'airat *et al*., 2021). The negative impact of free radical activity in the body can be inhibited by consuming antioxidants. Antioxidants are electron-donating compounds or reducing agents. These compounds can inhibit oxidation reactions by preventing the formation of free radicals or by binding to free radicals that have been formed, and consequently, cell damage will be inhibited (Sredoja *et al*., 2021; Yan *et al*., 2020; Annunziata *et al*., 2018; Wang *et al*., 2020).

Tea has long been known to the world community as a drink with distinctive taste and health benefits. The health benefits of tea consumption have been recognized for thousands of years in China and other countries. The development of cultivation techniques and tea leaf processing methods has led to more and more diverse types of tea, including black tea, green tea, and white tea (Zhao *et al*., 2019; Hajiaghaalipour and Sanusi, 2016). Tea leaves (*Camellia sinensis*) have long been consumed by the general public as a beverage that contains strong antioxidants. Based on the processing method, tea is classified into fermented tea (black tea), semi-fermented (oolong tea), and non-fermented (green tea). The tea processing process is further diversified into several special processing types, including white tea (Chen *et al*., 2020; Pérez-Burillo *et al*., 2018).

White tea has recently become famous and received special attention because it is predicted that white tea's antioxidant activity is greater than other types of tea (Li *et al*., 2020). White tea is made from very young tea leaves or from buds that are still closed, and there are silvery-white hairs or feathers (Pan *et al*., 2018). The younger the tea leaves, the higher the polyphenol as antioxidant compounds (Zhao *et al*., 2019). Research on white tea has been conducted by comparing the antimicrobial activity of white tea, green tea, and black tea against *Streptococcus mutans* bacteria (Utami, 2011).

Based on previous studies, it is necessary to do further research on the antioxidant activity of white tea by using extraction methods other than brewing. The extraction used in this study was maceration, then continued by fractionation using a liquid-liquid extraction method for the extract of white tea leaves. The extracts and fractions of white tea leaves obtained were then tested for their antioxidant activity by observing the ability of the extracts and fractions to scavenge free radicals 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH). The activity of the sample is expressed in IC_{50}, which is the concentration required to inhibit 50% of the absorption intensity after being compared with the blank (Molyneux, 2004; Rajasree *et al*., 2021; Munteanu and Apetrei, 2021). Furthermore, determining total polyphenol levels in white tea leaves was carried out to see the correlation between antioxidant activity and polyphenol levels in white tea leaves.

Materials and Methods

Chemicals and reagents

The white tea leaves used as samples in this study were obtained from the Tea and Cinchona Research Center Gambung, Ciwidey, West Java Indonesia and were analysed to prove their authenticity at the School of Life Sciences and Technology, Institut Teknologi Bandung West Java, Indonesia. The determination result stated that the plant used was *Camellia sinensis* (L.) Kuntze with letter number 214. II.CO2.2.2015.

Chloroform, 96% ethanol, ammonia, hydrochloric acid, amyl alcohol, toluene, ether, sulfuric acid, potassium hydroxide, sodium hydroxide, $FeCl_3$, $AlCl_3$ and silica gel thin-layer plate F_{254} were purchased from Merck (Jakarta, Indonesia). The reference standard for quercetin, 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), tannic acid and Folin Ciocalteu's reagent, was purchased from Sigma (St. Louis, MO, USA).

Plant identification

Taxonomic identification of tea plant (*Camellia sinensis* L.) was carried out at the School of Life Sciences and Technology, Institut Teknologi Bandung Indonesia.

Processing and storage of plant material

The collected plant material was then stored in an airtight container and mashed to obtain dry material powder. All dry material was ground into powder and stored in a clean and closed plastic container.

Standardization of white tea

Determination of dry material characteristics: Standardization of white tea plant includes macroscopic, microscopic analysis, determination of total ash content, water-soluble ash, acid insoluble ash, water content, lost on drying loss, water-soluble

and ethanol-soluble materials (Anonymous, 2017).

Phytochemical screening

Phytochemical screening was carried out to determine the secondary metabolites in dry material and white tea extracts (Anonymous, 2017; Faramayuda *et al*., 2021a). The groups observed were alkaloids, polyphenols, tannins, monoterpenes, quinones, and steroids.

Extraction

A total of 300 g of white tea leaf dry material powder was placed into a macerator. Ethanol solvent was added until the dry material powder was completely submerged and left for 24 hours. During the immersion, the mixture was stirred several times so that the compounds contained in the dry material of white tea leaves could dissolve completely. After 24 hours, the filtrate was filtered. The residue was macerated again in the same way. This process was repeated five times until a clear filtrate was obtained. The total solvent used was 7800 mL. The obtained filtrate was collected as to total extract (ethanol extract). The extract was evaporated using a rotary evaporator (a rotary vacuum evaporator) to obtain a concentrated extract.

Fractionation

Ethanolic extract (40g) was dissolved in 100 mL of water-ethanol (8:2) and extracted with 100 mL of n-hexane (1:1) until completely extracted to obtain n-hexane and water fractions. The water fraction was re-extracted with 100 mL of ethyl acetate (1:1) until it was completely extracted to obtain a water fraction and an ethyl acetate fraction.

Qualitative antioxidant activity test

Free radical scavenging screening was carried out using the principle of the dynamolysis method (Rohdiana *et al*., 2013). The thick sample (extract and fraction) was diluted first and then dripped onto a watch glass. Then on top of the petri dish, filter paper is placed in a circle, equipped with an axis of paper at the circle's core. Let it rise in a circle until the filter paper dries. It is then sprayed with 0.1% w/v DPPH reagent. Set aside for 30 minutes. Extracts and free radical scavengers will show yellow spots on a purple background.

Thin layer chromatography (TLC) profile examination of the fraction with the best antioxidant activity

White tea leaf's ethyl acetate fraction was then examined for thin-layer chromatography (TLC) as the fraction with the best activity (Anonymous, 2017; Faramayuda *et al*., 2021b, 2022). The plate used is a silica gel plate F 254. The plate is given the upper and lower limits first, then the ethyl acetate fraction is highlighted at the bottom of the TLC plate. The plate is inserted into a chamber containing toluene: Ethyl acetate (5:5), which has been saturated. The eluent will rise to the upper limit. Then the TLC plate is removed from the chamber and dried. Observation of the eluted TLC plate was carried out using an ultraviolet (UV) lamp with a short wavelength (254 nm) and a long wave UV lamp (365 nm).

Determination of total polyphenol levels

Preparation of tannic acid standard curve (Ministry of Health of the Republic of Indonesia, 2011): The tannic acid was prepared in methanol at a 5, 15, 30, 50, 70, 100 µg/mL concentration series. Take 1 mL plus 5 mL of Folin Ciocalteu's. Let stand for 8 minutes, add 4 mL of 1% NaOH and then incubate for 1 hour. Absorption was measured at a maximum wavelength of 779 nm with a UV-Visible spectrophotometer.

Measurement of total polyphenol content in white tea leaves (Ministry of Health of the Republic of Indonesia, 2011): The solution was made at a concentration of 1%. Take 1 mL plus 5 mL of Folin Ciocalteu's. Let stand for 8 minutes, add 4 mL of 1% NaOH, and incubate for 60 minutes. Absorption was measured at a maximum wavelength of 779 nm with a UV-Visible spectrophotometer. Measurements were carried out three times.

Results and Discussion

A macroscopic examination was carried out to introduce the material used to determine the identity (Figure 1) (Faramayuda *et al*., 2021c). Microscopic examination was carried out on dry material powder and fresh leaves to know the shape of specific fragments. From the results of microscopic examination with 400x magnification, it was seen that there were anomocytic type stomata, polygonal-shaped epidermal tissue, and the dry material powder contained xylem transporting bundles with spiral thickening (Figure 2).

Determination of the water content of dry material was carried out by the distillation method. The literature states that the water content requirements

for good dry material are below 10% (Anonymous, 2017). Determination of water content aims to determine the water contained in dry material, because high water content can cause dry material to be contaminated by microorganisms or bacteria. The results of the water content in dry material white tea leaves have met the requirements of 2% v/w. So that contamination of microorganisms such as the growth of fungi or microbes can be prevented (Table 1).

Figure 1: *Macroscopic leaves and white tea dry material. White tea leaves (A), dry material white tea leaves (B).*

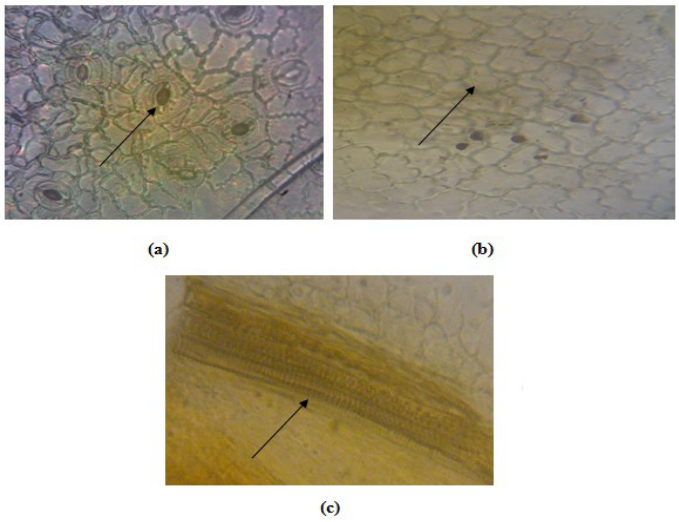

Figure 2: *Microscopic examination of white tea leaves and white tea dry material 400x magnification. Stomata of anomocytic type, longitudinal leaf incision (a), polygonal epidermal tissue, longitudinal leaf incision (b), xylem carrier bundle with spiral thickening, dry material powder (c).*

Table 1: *Characteristics of white tea leaf dry material.*

No	Parameter	Results
1	Total ash content (% w/w)	2.71±0.29
2	Water soluble ash content (% w/w)	2.06±0.021
3	Acid insoluble ash content (% w/w)	0.36±0.028
4	Water soluble extract content (% w/w)	35.23 ± 0.25
5	Ethanol soluble extract content (% w/w)	29.40 ± 0.45
6	Moisture content (% v/w)	2.00±0

Water-soluble ash content and acid insoluble ash content were determined to measure the mineral content and metal contamination contained in white tea leaf dry material, including physiological ash (ash originating from the plant itself) and non-physiological ash (ash originating from external contamination). Such as water, air and soil pollution). The total ash content obtained from white tea leaf dry material was 2.70 ± 0.27% w/w. These results indicate the amount of inorganic compounds in white tea leaf dry material. The results of the total ash content of white tea leaves have met the requirements of the Indonesian Herbal Pharmacopoeia, which is not more than 5.6% (Anonymous, 2017). The water-soluble ash content of white tea leaf dry material was 2.06 ± 0.021% w/w. This water-soluble ash content indicates the presence of water-soluble salts contained in the dry material.

The acid insoluble ash content of dry material in white tea leaves was 0.36 ± 0.028 % w/w. The results of the acid insoluble ash content of dry material in white tea leaves have met the requirements of the Indonesian Herbal Pharmacopoeia, which is not more than 0.6%. Determination of acid-insoluble ash content aims to indicate the presence of silica compounds in dry material. The results obtained from the determination of water-soluble and acid-insoluble ash content in white tea leaf dry material showed that the silica content in white tea leaf dry material was smaller than the water-soluble salt content. Silica is a contaminant that comes from outside, such as contamination of water and soil around where plants grow, which is then absorbed by plants or comes from air pollution.

The determination of the extractable material was carried out using two types of solvents, namely water and ethanol. Determination of water-soluble extract content and ethanol-soluble extract content was carried out to determine how much compounds were contained in dry material. In white tea leaf dry material, the water-soluble content was 35.24±0.25% w/w, and the ethanol-soluble content of white tea leaf dry material was 29.40±0.44% w/w. The determination of the dry material extract of white tea leaves showed that the compounds dissolved in water were more than the compounds dissolved in ethanol (Table 1).

Phytochemical screening of white tea leaf dry material was carried out to determine secondary metabolite compounds contained in white tea leaf dry material. The results of phytochemical screening showed that white tea leaf dry material contained flavonoids, polyphenols, quinones, alkaloids, tannins, monoterpenes and sesquiterpenes (Table 2).

Table 2: *Phytochemical screening of dry material, extract, and white tea fractions.*

Compound Group	Dry Material	Ethanol extract	Fraction N-hexane	Ethyl Acetate Fraction	Water faction
Alkaloids	+	+	+	+	-
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+
Polyphenol	+	+	+	+	+
Saponins	-	-	-	-	-
Quinone	+	+	+	+	+
Steroids and triterpenoids	-	+	+	-	-
Monoterpenoids and Sesquiterpenoids	+	+	+	+	+

Information: (+) Indicates the compound tested positive (-) Indicates the compound tested negative.

Maceration is the extraction method used in this study. Maceration is a cold extraction method used to avoid damage to thermolabile compounds in dry material. Maceration was done by immersing dry material in 96% ethanol solvent. Repeat several times until the maceration solvent is clear. Clear maceration solvent signifies that the substances or secondary metabolites contained in dry material have been extracted completely. The concentrated extract from the dry material maceration of white tea leaves was then evaporated to obtain a thick extract. The yield of the extract obtained was 40.82%.

Fractionation aims to separate the content of compounds based on their level of polarity. The separation process can occur due to the distribution of compounds in the extract or material into two solvents that do not mix. The solvents used in this fractionation process have different levels of polarity, where water is used as a polar solvent, ethyl acetate as a semipolar solvent, and n-hexane as a non-polar solvent. When fractionated, the ethanol extract of white tea leaves was first dissolved in water-ethanol (8:2 v/v). Ethanol was used to help dissolve the extract in water when it was fractionated from the results of the fractionation produced fractions with different levels of polarity, namely the water fraction, the ethyl acetate fraction, and the n-hexane fraction. The results of the fractionation of white tea leaf ethanol extract, the ethyl acetate fraction, was the fraction with the largest yield with a yield of 72.94%, while the n-hexane fraction was the fraction with the smallest yield of 4.2%, for the water fraction itself, the percentage yield was 17.08 %. From these results, it can be seen that the compounds contained in white tea leaves are mostly semipolar (Anonymous, 2017; Faramayuda *et al*., 2021; WHO, 2017).

The first antioxidant activity test was carried out qualitatively with the dynamolysis method. Qualitative testing was carried out on the ethanol extract and white tea leaf fractions. From the qualitative test by dynamolysis, it was expected that a reaction would occur between the sample and DPPH as a free radical. The tested sample was spread evenly on filter paper and then sprayed with 0.1% w/v DPPH solution. Samples of both extracts and fractions of white tea leaves showed a colour change where the reaction results showed a yellow colour with a purple background on the filter paper, indicating that the sample tested positive had antioxidant activity (Table 3).

Table 3: *Screening of dry material free radical capture activity of white tea leaves (Camellia sinensis L.).*

No	Sample	Results
1	Ethanol extract	+
2	Ethyl acetate fraction	+
3	n-hexane fraction	+
4	water fraction	+

Information:(+) provides radical scavenging activity free. (-) does not provide free radical scavenging activity.

Antioxidant activity of white tea leaves was quantitatively carried out using 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) reagent against ethanol extract and white tea leaf fractions. (Molyneux, 2004; Rajasree *et al*., 2021; Munteanu and Apetrei, 2021). Measurements were made using an ultraviolet (UV) spectrophotometric instrument. The maximum wavelength of the DPPH solution at 30 µg/mL in methanol was 515.50 nm (Figure 3). The antioxidant activity of white tea leaves is expressed in the IC_{50} value, which is a concentration that can inhibit 50% of free radicals compared to the DPPH reagent solution. The IC_{50} value is calculated from the linear regression equation between the concentration of the test solution and the percent reduction. The greater the concentration of the solution, the higher the percent reduction because the concentration of the test solution is directly proportional to the percent reduction (Molyneux, 2004).

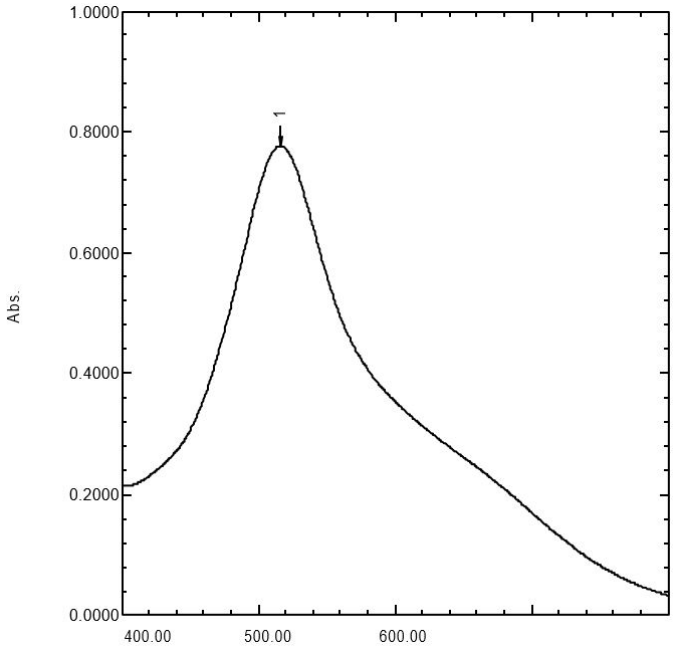

Figure 3: *Absorption curve of DPPH solution. Reagent solution = DPPH solution 30 µg/mL in methanol at a wavelength (λ) max 515.50 nm with a maximum absorption of 0.778.*

Table 4: *Free radical capture test results comparison of quercetin, extract and fraction – white tea leaf fraction.*

Sample	IC50 (µg/mL)
Quercetin	1.05
Ethanol extract	2.03
Water fraction	2.17
Ethyl acetate fraction	1.55
n-hexane fraction	4.77

The higher the concentration, the purple colour produced by DPPH free radicals will be further reduced by the test solution containing antioxidant compounds. As a standard in the quantitative antioxidant activity test, quercetin was used as a standard. Quercetin was used as a standard because quercetin is an isolate derived from plants and has been known to have excellent antioxidant activity. IC_{50} of ethanolic extract of white tea leaves was 2.03 µg/mL, IC_{50} of water fraction of white tea leaves was 2.17 µg/mL. IC_{50} of white tea leaf n-hexane fraction was 4.77 µg/mL, and IC_{50} of ethyl acetate fraction was 1.55 µg/mL. For standard, quercetin has an IC_{50} of 1.05 µg/mL. From the results of this quantitative antioxidant activity test, it is known that the IC_{50} value of the ethyl acetate fraction is the smallest compared to the ethanol extract, the water fraction, and the n-hexane fraction of white tea leaves. Meanwhile, the fraction that has the largest IC_{50} value is the n-hexane fraction with an IC_{50} value of 4.77 µg/mL (Table 4; Figure 4).

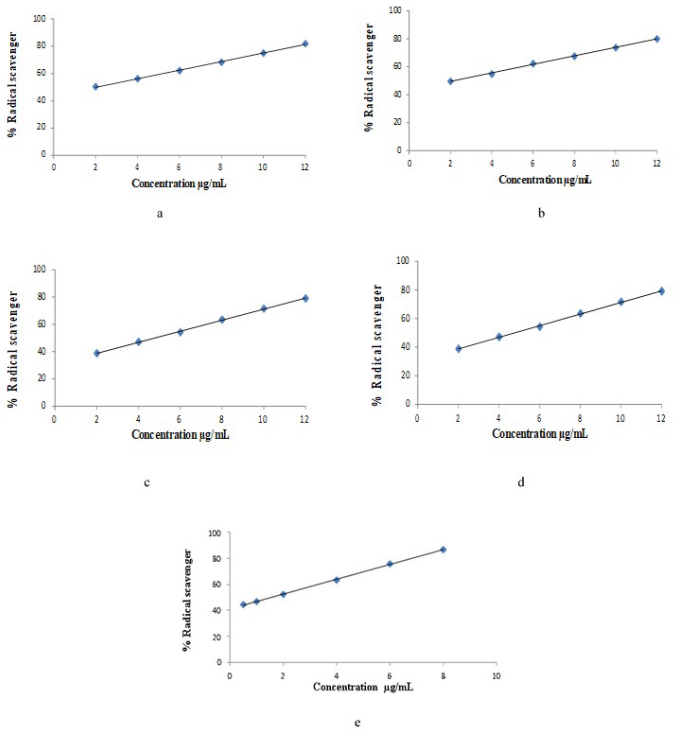

Figure 4: *Linear regression curve of DPPH free radical scavenging percentage. quercetin (a), ethanol extract (b), water fraction (c), n-hexane fraction (d), ethyl acetate fraction (e).*

For antioxidant research, the antioxidant activity of white tea has been tested using the DPPH method based on temperature and brewing time. The temperatures used were 55°C, 75°C, and 95°C, with 3 minutes, 5 minutes, and 9 minutes of brewing time. The results of this study prove that white tea has good antioxidant activity. This is indicated by the low IC_{50} value at 95°C and the brewing time for 9 minutes of 35.41 µg /mL with the highest total polyphenol content of 6.01% (Rohdiana *et al.*, 2013).

The higher the IC_{50} value, the weaker the antioxidant activity. On the contrary, the smaller the IC_{50} value, the stronger the antioxidant activity. Based on the screening results, almost all compounds were positive except for steroids and triterpenoids, and for the IC_{50} value, the ethyl acetate fraction had an IC_{50} value of 1.55 µg/mL, almost equivalent to the IC_{50} value of quercetin 1.05 µg/mL. From the results of the IC_{50} value, it can be said that the ethyl acetate fraction has the best antioxidant activity compared to other extracts and fractions. The ethyl acetate fraction belongs to a very strong antioxidant group because it has an IC_{50} value < 50 µg/mL (Molyneux, 2004; Rajasree *et al.*, 2021; Munteanu and Apetrei, 2021).

The total polyphenol content of white tea leaves was 0.373±0.0064% (Table 5). polyphenolic compounds

are secondary metabolites widely contained in white tea and have been known to have good antioxidant activity with high polyphenol content (Paiva *et al.*, 2021).

Table 5: *The results of the measurement of the total polyphenol content of white tea leaves.*

No	Absorption	Rate (%)
1	0.533	0.366
2	0.547	0.376
3	0.550	0.378
Average		0.373
SD		0.0064

Polyphenol compounds that act as antioxidants in tea include catechins, epigallocatechin 3-gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), and epicatechin 3-gallate (ECG). The mechanism of polyphenolic compounds contained in white tea as antioxidants in reducing free radicals 1, 1-Diphenyl-2-Pikrylhydrazil (DPPH) is by donating or giving hydrogen to DPPH free radicals. The nature of free radicals, which were initially unstable, became stable after obtaining hydrogen from polyphenolic compounds of white tea leaves such as catechins, epigallocatechin gallate, epigallocatechin, epicatechin, and epicatechin gallate. So that the free radical DPPH becomes stable and the activity of free radical compounds can be inhibited (Rajasree *et al.*, 2021; Munteanu and Apetrei, 2021).

Polyphenols act as antioxidants or scavenge free radicals through four mechanisms: Damaging free radicals as hydrogen donors. To prevent the formation of free radicals, deactivate single oxygen, which acts as a free radical, and capture metals, namely by binding to metals that can inhibit the formation of free radicals. The ability to scavenge free radicals by polyphenolic components can also be seen as the ability to donate hydrogen (Zhao *et al.*, 2019). White tea also has the potential to lower cholesterol levels (Luo *et al.*, 2020), antidiabetic (Xia *et al.*, 2020), anti-cancer (Bondarian *et al.*, 2019; Liu *et al.*, 2018), anti-bacteria (Kusumawardani *et al.*, 2019), neuroprotective (Li *et al.*, 2019) and overcome liver function disorders (Wang *et al.*, 2019).

Then the thin layer chromatography (TLC) pattern was examined. The TLC plate mobile phase consists of a mixture of two solvents with different polarity properties so that the chemical composition of the fractions can be separated. The TLC results of the n-hexane fraction, when observed under a 365 nm UV lamp, appeared reddish-orange spots with Rf 0.66, 0.77, 0.88 and 0.93, observations under UV lamp at wavelength 254nm there was a black spot at Rf 0 .66, 0.88 and 0.93. After being sprayed using the $AlCl_3$, it was observed again under a 365 nm UV lamp, the spot changed colour to blue fluorescence at Rf 0.44, 0.66, 0.77, 0.88 and 0.93. When sprayed with $FeCl_3$ spots, black spots appeared, visually observed at Rf 0.88 and 0.93 (Figure 6). Spots that reacted positively with $FeCl_3$ indicated the suspected presence of phenolic compounds in white tea leaves (Anonymous, 2017).

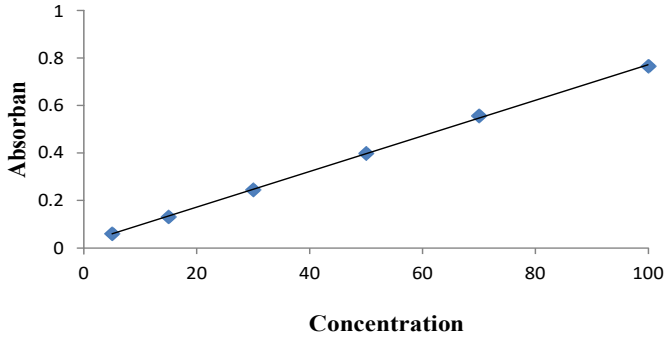

Figure 5: *Tannic acid calibration curve at λ 779 nm.*

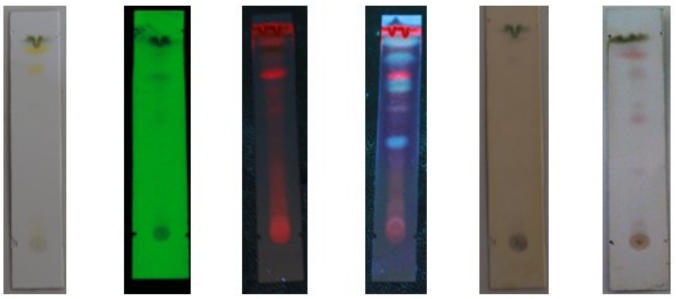

Figure 6: *Thin layer chromatography of white tea leaf n-hexane fraction. Mobile phase: Chloroform: Methanol (9:1). visually observed (a); observed under 254 nm ultraviolet light, the presence of black spots with Rf 0.66, Rf 0.88 and Rf 0.93 (b); observed under 365 nm ultraviolet light, the presence of blue spots at Rf 0.66, Rf 0.77, Rf 0.88, and Rf 0.93 (c); sprayed with the sight of AlCL3 spots, the spots turned bright blue at Rf 0.44, Rf 0.67, Rf 0.77, Rf 0.88, and Rf 0.93 (d); used FeCl3 spot viewer, black spots appear with Rf 0.88 and 0.93 (e); given the appearance of vanillin sulfate spots, purple spots appeared at Rf 0.33, Rf 0.6, Rf 0.67, Rf 0.82 and Rf 0.88 (f).*

The stationary phase used is cellulose, and the mobile phase is butanol: acetic acid: water (BAW) using a mobile phase ratio of 4:1:5. There is a long black spot when observed under a 254 nm UV lamp. The chromatographic pattern of the water fraction was

viewed under a 365 nm UV lamp, blue spots were observed. Then given the sight of $AlCl_3$ and ammonia reagent (WHO, 2017). When given the $AlCL_3$ reagent, the spot changes colour to bright blue, and after being given ammonia reagent, the spot changes colour from blue to yellowish green at Rf 0.62 (Figure 7).

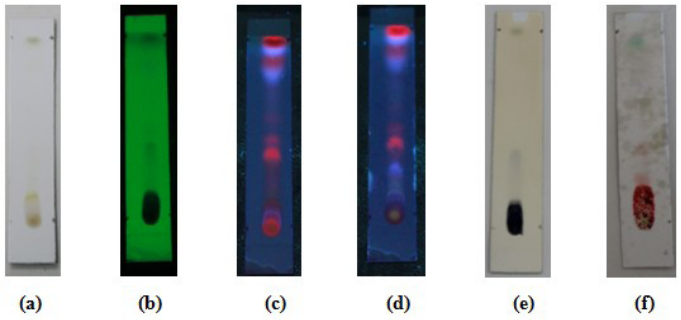

Figure 7: *Thin layer chromatography of white tea leaf ethyl acetate fraction. Mobile phase: Toluene: Ethyl Acetate (5 : 5). Visuals (a); observed under 254 nm ultraviolet light, the presence of black spots with Rf 0.44 and Rf 0.51 (b); observed under 365 nm ultraviolet light, the presence of reddish orange spots at Rf 0.44, Rf 0.51, and the presence of blue spots at Rf 0.77 and 0.93 (c); sprayed with AlCL3 spot sight, the spots changed to a bright blue color at Rf 0.27, Rf 0.66, Rf 0.77, and Rf 0.93 (d); used FeCl3 spot viewer, blue-black spots appear with Rf 0.44 (e); Vanillin sulfate was used to show spots, light purple spots appeared at Rf 0.27, Rf 0.4, and blue spots appeared at Rf 0.93 (f).*

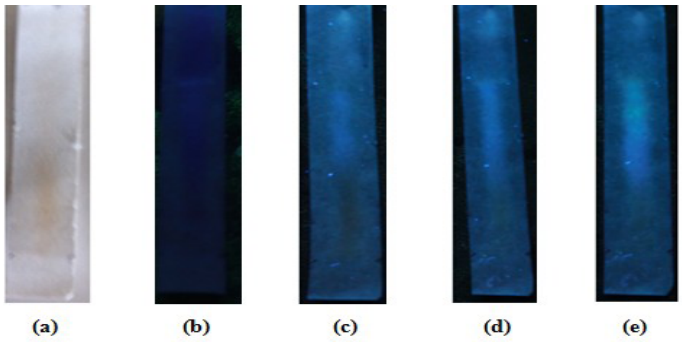

Figure 8: *Paper chromatography of white tea leaf water fraction. Mobile phase: Butanol: Acetic Acid Water (4 : 1 : 5). Visual(a); observed under a 365 nm UV lamp (b); observed under 365 nm ultraviolet light, the presence of a tailed blue spot (c); sprayed with AlCL3 spot sight and observed under 365 nm ultraviolet light, a bright blue colored spot appears with tail (d); after being given ammonia vapor and observed under ultraviolet light at 365 nm, a yellowish green spot appeared at Rf 0.62 (e).*

The mobile phase used in the ethyl acetate fraction of white tea leaves is toluene: Ethyl acetate (5:5). The TLC results for the ethyl acetate fraction were viewed under a UV lamp with a wavelength of 365 nm, reddish-orange spots appeared at Rf 0.44 and 0.51, and blue fluorescence spots had Rf 0.77 0.93. Meanwhile, when viewed under a 254 nm UV lamp, a black spot appears with Rf values of 0.44 and 0.51. The appearance of $AlCl_3$ spots showed a change in spot colour from orange to blue fluorescence, which was seen under a 365 nm UV lamp at Rf = 0.44, 0.66, 0.77, and 0.93. When given the visual appearance of $FeCl_3$ spots on the plate, a black spot was seen at Rf 0.44. Observations for monoterpenes and sesquiterpenes were carried out visually. The plate was sprayed with vanillin sulfate spots. A light purple spot appeared at Rf 0.27, Rf 0.4, and a blue spot at Rf 0.93. The spot colour changed from colourless visually to pink and blue (Figure 8).

Conclusions and Recommendations

Anntioxidant activity of the ethanol extract, n-hexane fraction, water fraction, ethyl acetate fraction and quercetin measured as IC_{50} were 2.03 µg/mL4.77 µg/mL; 2.17 µg/mL; 1.50 µg/mL and 1.07 µg/mL., respectively. The ethyl acetate fraction has antioxidant activity equivalent to quercetin and can be categorized as a very strong antioxidant. White tea leaves have a total polyphenol content of 0.373±0.0064%. Based on thin-layer chromatography (TLC) results, the compounds thought to provide antioxidant activity are polyphenols and flavonoids.

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Novelty Statement

The results of this study provide new information about the antioxidant activity of the ethyl acetate, water and n-hexane fractions of white tea leaves.

Author's Contribution

Fahrauk Faramayuda, Soraya Riyanti and Sitty Mahanadhiandinie carried out the experiment and wrote the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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