

Analysis of Caffeine, Tannin and Total Phenol Content of Tea Leaves from Sirah Kencong Blitar Plantation

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Abstract

Caffeine, tannins, and total phenol are chemical components of tea leaves that are beneficial for the body. Caffeine is a psychoactive substance, tannin is anti-diarrhea, and phenol compounds are antioxidants that prevent free radicals. The purpose of this research was to analyze the levels of caffeine, tannin, and total phenol of tea leaves at Sirah Kencong Blitar Plantation. Determination of the caffeine level was carried out in tea leaves powder using UV-Vis Spectrophotometry method at 273.5 nm wavelength with caffeine as a standard. The determination of tannin and total phenol levels was carried out using the ethanol extract of tea leaves. Tannin was determined by the Follin Denis method using UV-Vis spectrophotometry at 649.9 nm wavelength with tannic acid as a standard. The total phenol was determined by the Follin Ciocalteu method using UV-Vis spectrophotometry at 794 nm wavelength with gallic acid as a standard. Based on the results of the research in tea leaves from the Sirah Kencong Blitar Plantation, the caffeine content was 1.649%, tannin was 7.095%, and total phenol was 34.142%.

Keywords: caffeine, tannin, total phenol, tea leaves

INTRODUCTION

The tea plant is a plant species used to make tea; the leaves and leaf shoots are heated to deactivate the enzymes in the tea leaves before being rolled and dried. There are three types of tea depending on how they are processed: fermented tea (black tea), semi-fermented tea (oolong tea), and unfermented tea (green tea) (Rohdiana, 2015). In addition to vitamins C and E, catechin, caffeine, polyphenols, theophylline, flavonoids, tannins, and several minerals like Zn, Se, Mo, Ge, and Mg, tea also includes beneficial components that serve as antioxidants, anti-mutagenic, and anti-cancer agents (Redjeki, 2015). Therefore, tea is not only a drink that can quench thirst but also has benefits for the health and fitness of the drinker (Abdolmaleki, 2016).

The chemical compound content in tea leaves can be classified into four large groups. There are the phenol group, non-phenol group, aromatic group, and enzymes. These four groups support the good properties of tea if control during processing can be carried out properly (Balitri & Towaha, 2013). Caffeine is a non-phenol group that is included in alkaloids. The refreshing properties of brewed tea come from the alkaloid compounds it contains, namely 3-4% of the dry weight of the leaves. Caffeine is a

psychoactive substance found in coffee, tea, and chocolate. Caffeine has pharmacological effects that are clinically useful, such as eliminating feelings of fatigue, hunger, and drowsiness as well as increasing concentration and strengthening heart contractions. Excessive caffeine consumption can cause heart palpitations, headaches, feelings of worry and anxiety, trembling hands, restlessness, reduced memory, and difficulty sleeping, and the acidic nature of the compound can cause stomach and digestive problems (Tjay & Rahardja, 2002).

The phenol group, or what can be called phenolic compounds, are compounds that have one or more hydroxyl groups attached to an aromatic ring (Proestos et al., 2006). The highest phenol content in tea is found in the leaves at 5-27% and fresh leaves at 36% (Paramita et al., 2020). Most of the benefits of polyphenols are antioxidants, so they can neutralize free radicals, which can damage body tissues and cells. The high antioxidant content can slow down the aging process. It can improve heart health and speed up blood circulation. It can also reduce the risk of cardiovascular disease and heart disease. Even though the existence of free radicals also has advantages for the human body, such as being able to help with the destruction of microorganism cells, cancer, and the process of maturation of cells in the body. Therefore, excess

antioxidant content can eliminate free radicals, thereby allowing bad effects on the body to occur if excess antioxidants are consumed (Paramita et al., 2020).

Tannins are a group of polyphenols. It has properties such as antidiarrheals, astringents, ulcers, and stop bleeding. Tannins also help neutralize fat in food and prevent oxidation of low-density fat, which can become plaque, lower blood cholesterol, freshen breathing, and stimulate the brain stem (Djamil, 2011). In tea plants, the highest levels of tannin are found in the tea shoots, so the shoots have high quality. The tannin content in tea can be used as a quality guide because tannins can provide a distinctive taste. The tannins in tea also have a bad effect on the body. Tannins play a role in reducing the absorption of iron (Fe). Consuming tea can reduce blood cells' iron absorption by as much as 64%, thereby triggering anemia. In addition, tannins are known to bind to proteins and minerals. So, proteins cannot be absorbed by the body (Fajrina et al., 2017).

The Sirah Kencong plantation is one of the tea-producing plantations in the Blitar area, precisely in Ngadirenggo Village, Wlingi District, Blitar Regency, 40 km from Blitar City. This 219.15-hectare plantation has an altitude of 1,000 meters above sea level. Moreover, by cultivating tea plants, Sirah Kencong Plantation also produces packaged black tea under the Ken Tea trademark. Before processing it into packaged tea, research needs to be carried out regarding the beneficial compounds contained in Sirah Kencong Plantation tea leaves so that they can be used as a comparison of the levels of beneficial compounds before and after the processing process.

Based on this background, researchers conducted research about "Analysis of Caffeine, Tannin and Total Phenol Content of Tea Leaves from Sirah Kencong Blitar Plantation". Analysis of caffeine, tannin, and total phenol levels needs to be carried out on tea leaves from Sirah Kencong Blitar Plantation, which can be used as a reference regarding the content of caffeine, tannin, and total phenol, considering that these compounds have many benefits for the body. Moreover, it can be used as an information source to determine the differences in caffeine, tannin, and total levels in tea leaves and packaged tea. The caffeine, tannin, and total phenol levels analysis was carried out using the UV-Vis spectrophotometer. UV-Vis spectrophotometer is a simple way to determine low quantities of substances. Furthermore, determining the content of the sample using a UV-Vis spectrophotometer is relatively easy, fast, and accurate (Fajrina et al., 2017). It is hoped that this research can be used as a source of information for future

researchers regarding the caffeine, tannin, and total phenol levels of tea leaves from the Sirah Kencong Blitar Plantation.

METHODOLOGY

Materials and Instrumentals

Materials used in this research are filter paper, tea leaves (Perkebunan Sirah Kencong), caffeine standard (Merck), tannic acid standard (Merck), gallic acid standard (Merck), chloroform (Merck), CaCO₃ (Merck), ethanol 96% (Merck), *Follin-Denis* reagent (Nitro Kimia), *Follin-Ciocalteu* reagent (Nitro Kimia), and Na₂CO₃ (Merck), aquadest.

The instruments used in this research are a UV-Vis Spectrophotometer (Jasco V-750), analytical balance (Shimadzu ATY224), vortex (IKA Vortex 3), separatory funnel (Iwaki), rotary evaporator (IKA RV 10), water bath (Memmer), graduated pipette (Pyrex), beaker glass (Iwaki), measuring cylinder (Pyrex) funnel glass (Herma), volumetric flask (Iwaki), stirring rod (Pyrex), dropping pipet (Pyrex), test tube (Iwaki), hot plate (Thermo Scientific Cimarec), spatula.

Procedures

A. The sample preparation (Mukhriani et al., 2014)

The tip tea leaves were picked and put in a plastic bag. Sorted and steamed at temperature 90°C for 2 minutes. Then dried at room temperature for 5x24 hours. After drying, it was mashed to get the tea leaves powder. 50 grams of tea leaves powder was macerated with 500 mL ethanol 96% for 3x24 hours. After that, it was filtered to obtain the extract (filtrate). The filtrate was evaporated in the rotary evaporator to obtain tea ethanol extract.

B. The Tannin determination (Irianty & Yenti, 2014)

1 mg of tea ethanol extract was weighed and dissolved in aquadest to 10 mL (100 ppm). 9 mL of the solution was pipetted and dissolved in aquadest to 10 mL (90 ppm). 1 mL of *Follin Denis* reagent was added and left for 3 minutes. 1.0 mL Na₂CO₃ saturated solution was added and incubated for 40 minutes. The absorption was measured using a UV-Vis spectrophotometer on wavelength 649,9 nm.

C. The phenol total determination (Kusmiyati et al., 2016)

10 mg of tea ethanol extract was dissolved in aquadest to 10 mL (1000 ppm). 0.5 mL of solution was pipetted and added with the aquadest until 5 mL (100 ppm). A solution is made with a dilution factor of 20 with pipetted 1,25 mL of the 100 ppm test solution and dissolved with aquadest in a 25 mL volumetric flask. 1

mL of solution test was pipetted, then added 0,5 mL *Follin-Ciocalteu* reagent was left for 8 minutes while stirred. 4 mL of Na₂CO₃ 7,5% was added and vortexed for 1 minute. The absorption was measured using a UV-Vis spectrophotometer on wavelength UV-Vis 794 nm.

D. The caffeine determination (Wardani & Fernanda, 2016)

1 gram of tea leaf powder was weighed, and then 150 mL of hot aquadest was added and brewed for 2 minutes while stirring. The hot tea solution was filtered. The filtrate of tea solution was added with 1.5 grams of CaCO₃ and extracted with chloroform as much as four times. 25 mL of chloroform was added at each extraction. The bottom layer (chloroform layer) was taken and evaporated using a rotary evaporator. The caffeine extract obtained was marked with distilled water in a 100 mL volumetric flask. Dilute 100 times in a 10 mL volumetric flask. The test solution was analyzed using a UV-Vis spectrophotometer on wavelength 273.5 nm.

E. The Tannic acid standard preparation (Irianty & Yenti, 2014)

10 mg tannic acid was weighed and dissolved with aquadest in a 10 mL volumetric flask. The standard solutions were made with concentrations of 10, 15, 20, 25, 30, and 35 ppm from this solution. 1 mL of each solution was taken and mixed with 1 mL of Follin Denis reagent. The mixture was left for 3 minutes. After that added with 1 mL of saturated Na₂CO₃ and placed in a place not exposed to light for 40 minutes for the homogenization process.

F. The gallic acid standard preparation (Kusmiyati et al., 2016)

10 mg gallic acid was weighed and dissolved with aquadest in a 10 mL volumetric flask. The standard solutions were made with concentrations of 10, 15, 20, 25, 30, and 35 ppm from this solution. 1 mL of each solution was taken and it was mixed with 0.5 mL of *Follin-Ciocalteu* reagent and left for 8 minutes while stirring. 4 mL of Na₂CO₃ 7,5% was added and vortexed for one minute.

G. Caffeine standard preparation (Wardani & Fernanda, 2016)

10 mg caffeine solids were weighed and dissolved with 10 mL of distilled water in a 10 mL volumetric flask. Mark and stir until homogeneous. A standard solution of 100 ppm caffeine was made. Then, a

standard caffeine solution of 2, 3, 4, 5, 6, and 7 ppm was made.

Data analysis

Data analysis was carried out by plotting the standard concentration data with the absorbance results data to obtain the linear regression equation (1). The standard data came from caffeine, tannic acid, and gallic acid standards. From the linear regression equation, the average concentration of the sample can be obtained based on the sample absorbance results, and then the levels of (2) caffeine, tannin, and total phenol in the sample are calculated.

$$y=ax+b \quad (1)$$

$$\%b/b = \frac{x \text{ (mg/L)} \times V \text{ solution(L)} \times fp \times 100\%}{\text{sample weight (mg)}} \quad (2)$$

RESULTS AND DISCUSSION

A. The sample preparation

Determination of caffeine, tannin, and total phenol content of tea leaves from the Sirah Kencong Blitar Plantation was carried out by hand-picking samples of tea leaf shoots from the Sirah Kencong Blitar Plantation. The top tea leaves are picked from 07.00 WIB to 09.00 WIB. The picked tea leaves are placed in a plastic bag. This procedure is to avoid oxidation when exposed to direct sunlight (Mutmainnah, Chadijah, & Qaddafi, 2018). The picked tea leaves are then sorted to separate the leaves from the stems and steamed at 90 °C for 2 minutes to deactivate the polyphenol oxidase enzyme, which can oxidize polyphenolic compounds in tea leaves to form theaflavin and thearubigin which results in a decrease in total polyphenols (Mukhrani et al., 2014). Then dried at room temperature for 5 days. Mabrurroh (2015). The drying process should be carried out at room temperature, namely less than 40 °C, because if it is more than that, there will be a drastic reduction in polyphenol levels. The dried tea leaves are ground using a blender to obtain tea leaf powder, as shown in Figure 1.

Table 1. The Results of Determination of Caffeine, Tannin, and Total Phenol Contents

Sample	Concentrate (ppm)	Absorbance	Content (%b/b)
Tea leaf (cafein)	1.0995	0.1317	1.65%
Tea leaf (tannin)	6.3855	0.5337	7.10%
Tea leaf (phenol total)	1.7071	0.0607	34.14%



Figure 1. Tea leaf sample

The determination of tannin and total phenol levels was carried out using the ethanol extract of tea leaves by macerating the extract on 50 grams of tea leaf powder using 500 mL of 96% ethanol solvent. 96% ethanol solvent can extract secondary metabolite compounds maximally. The large number of hydroxy groups in polyphenolic compounds causes these compounds to be polar and can dissolve in ethanol or water (Sari, 2010). The maceration process is carried out in a closed container for 3x24 hours, with stirring every 24 hours. After the maceration process is complete, filtering is carried out to separate the filtrate and maceration residue. The filtrate obtained was evaporated using a rotary evaporator until all the solvent had evaporated and an ethanol extract of tea leaves was formed.

B. Caffeine Determination

1 gram of tea leaf powder is brewed with 150 mL of hot distilled water for 2 minutes to dissolve the caffeine. Caffeine is soluble in water, alcohol, or chloroform but less soluble in ether. Solubility increases in hot water or hot alcohol (John M. Beale Jr John Block, 2011). Next, it is filtered to separate the filtrate and brewing residue. The brewing filtrate is added with 1.5 grams of CaCO_3 . The CaCO_3 function is to break the bonds of caffeine with other compounds so that the caffeine will be in the free base. This condition will be bound by chloroform during liquid-liquid extraction (Misfadhila, Zulharmita, & Siska, 2017). Next, the filtrate is put into a separating funnel for liquid-liquid extraction with chloroform. Extraction was carried out 4 times by adding 25 mL of chloroform to the water layer at each extraction so that the caffeine could be completely extracted. Next, two layers will be formed. It was the water layer and the chloroform layer. Chloroform, which is nonpolar, binds caffeine contained in the water layer and is in the bottom layer because it has a greater specific gravity. The water layer is on top because it is polar and has a smaller density. The chloroform layer from each extraction was mixed and evaporated over a water bath at 61 °C to obtain caffeine extract, which was then dissolved

with distilled water in a 100 mL volumetric flask. 1 mL of the solution was pipetted and diluted with distilled water in a 10 mL volumetric flask. Next, 1 mL of the solution was pipetted again and diluted with distilled water in a 10 mL volumetric flask to obtain a test solution with a 100 times dilution.

Determination of caffeine levels in tea leaves was carried out using caffeine standards as a comparison. A mother liquor of 1000 ppm caffeine was made, then a standard solution of 100 ppm caffeine and a standard caffeine solution were made with concentrations of 2 ppm, 3 ppm, 4 ppm, 5 ppm, 6 ppm, and 7 ppm. Determination of caffeine levels using a UV-Vis spectrophotometer was carried out at a wavelength of 273.5 nm (Mutmainnah et al., 2018). Measurements were carried out on standard solutions so that concentration and absorbance data were obtained and then plotted into a standard curve.

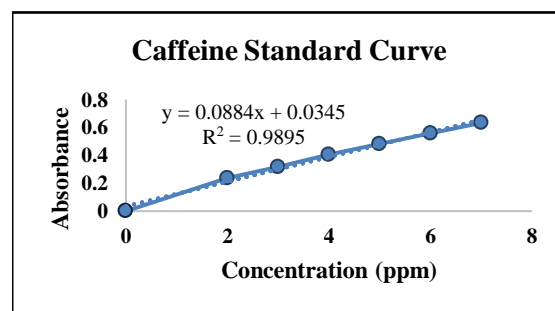


Figure 2. The Caffeine Standard Curve

Based on the standard curve, the equation obtained is $y = 0.0884x + 0.0345$ with $R^2 = 0.9895$. This equation is linear, so it can be used to determine caffeine levels in samples. Next, measurements were carried out on the sample, and the results were obtained from sample absorbance. From equation $y = 0.0884x + 0.0345$, the sample concentration can be calculated based on the sample absorbance results. After the calculations, the caffeine concentration in the sample was obtained at 1.0995 ppm. Next, the caffeine levels were calculated (%b/b), and the results were 1,649%. Based on research conducted by Francis dan Roberts to determine the caffeine content in *Camellia sinensis*, the results obtained ranged from caffeine content from black tea (1.8-4.6%), green tea (1.0-2.4%), and instant tea (4.0-5.0%) (Sutipno, 2019). The caffeine content resulting from this research was 1.649%, which is in the range of green tea caffeine content of about 1.0-2.4%. The tea leaves were analyzed when they did not undergo a fermentation process under green tea processing. Finally, the caffeine levels produced were relatively lower when compared to black tea and instant tea. The important role of caffeine in tea can determine the bitter/astringent taste of tea, but high

content caffeine in tea leaves is less desirable because its pharmacological properties can stimulate the central nervous system, so tea consumption must be regulated as in SNI 01-7152-2006, which states the maximum limit of caffeine in tea. Food and drink are 150 mg/day and 50 mg/serving. The amount of caffeine content in tea leaves can be caused by many factors. The type of tea leaves is one reason. It also comes from where the tea plant grows, the size of the tea particles, the method, and the brewing time (Artanti, Nikmah, & Setiawan, n.d.).

C. The tannin determination

1 mg of ethanol extract of tea leaves was dissolved in distilled water in a 10 mL volumetric flask to obtain a sample solution with a concentration of 100 ppm. Tannins are complex polyphenolic compounds that can dissolve in various polar solvents such as distilled water, ethanol, methanol, and acetone (Ismarani, 2013). 9 mL of the 100 ppm sample solution was pipetted and dissolved with distilled water in a 10 mL volumetric flask to obtain a sample solution with a concentration of 90 ppm. The sample solution was added with 1 mL of Folin Denis reagent and left for 3 minutes. The tannin in the sample will react with a color-forming reagent, Folin Denish reagent, based on a redox reaction. Tannin acts as a reducing agent, while Foline Denish's reagent acts as an oxidizing agent. Oxidized tannin compounds will change the phospholybdate in folin denish into blue phospholybdenum. It can absorb light in the visible ultraviolet wavelength region (Andriyani, Utami, & Dhiani, 2010; Istyami et al., 2024). Next, 1 mL of saturated Na_2CO_3 was added to form an alkaline atmosphere so that the Folin denish reduction reaction occurred by the hydroxyl groups of the polyphenols in the sample and would form a blue molybdenumtungsten complex. Then let it sit for 40 minutes for the homogenization process.

Determination of tannin levels in tea leaves using tannic acid as a comparison. Tannic acid is a group of hydrolyzed tannins. So, it can be used as a comparison in measuring total tannin levels (Istyami et al., 2024). A stock solution of 1000 ppm tannic acid and a tannic acid standard solution of 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm, and 35 ppm were prepared. 1 mL of each standard solution was pipetted and added with 1 mL of Follin Denis reagent. The mixed solution was left for 3 minutes, and 1 mL of saturated Na_2CO_3 was added. Next, leave it for 40 minutes in a place not exposed to light for the homogenization process.

Determination of tannin content using a UV-Vis spectrophotometer was carried out at a wavelength of

649.9 nm (Pratama dkk, 2019). Measurements were carried out on standard solutions. So, the data were

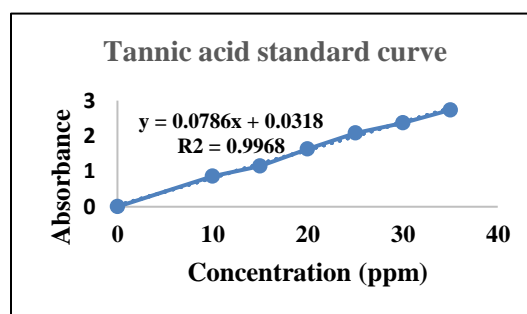


Figure 3. The tannic acid standard curve

obtained and then plotted into a standard curve.

Based on the standard curve, the equation obtained $y = 0.0786x + 0.0318$ with $R^2 = 0.9968$. This equation is linear so it can be used to determine the tannin levels of the samples. Next, measurements were carried out on the sample, and the results were obtained from sample absorbance. From equation $y = 0.0786x + 0.0318$, the tannin concentration in the sample can be calculated based on the sample absorbance results. The tannin concentration in the sample was obtained at 6.3855 ppm. Next, the tannin content was calculated (%b/b), and the results were 7.095%. Tannin is an active secondary metabolite compound optimally contained in tea leaves. Tea leaves contain about 5-15% tannin compounds. The tannin concentration in green tea leaves is 5-10% (Fajrina et al., 2017). In this research, the tannin content was 7.095%. These levels are in the range of tannin compound levels according to research conducted by Fajrina (2007). The tannin content obtained from green tea leaves can be influenced by the type of tea shoots used as research samples. In tea processing, tannin compounds influence the properties of the tea produced, namely taste, color, and aroma.

D. The total phenol determination

10 mg of tea leaves ethanol extract was dissolved in distilled water in a 10 mL volumetric flask to obtain a sample solution concentration of 1000 ppm. The solvent used for the total phenol test is distilled water because phenol compounds are polar, so they tend to dissolve in polar solvents (Anwariyah, 2011; Purwaningsih, Fathiah, Amaliyah, & Kuswiyanto, 2023). The 1000 ppm sample solution was pipetted in 0.5 mL and diluted with distilled water in a 5 mL volumetric flask to obtain a sample solution with a concentration of 100 ppm. Next, a solution was made with a dilution factor of 20 times by pipetting 1.25 mL of 100 ppm sample solution and diluting it with distilled water in a 25 mL volumetric flask. 1 mL of the

solution was taken and put into a test tube. Next, 0.5 mL of Follin Ciocalteu reagent was added and left for 8 minutes while shaking. Follin Ciocalteu reagent is a complex solution formed from heteropolyphosphotungstic acid phosphomolybdic acid, which can measure all phenolic compounds in the test sample. Follin Ciocalteu is yellow and will change color to blue. Phosphomoblid oxidizers will react with phenolic compounds to produce molybdenum-tungsten complexes and blue phenolic compounds. The more intense the color produced, the higher the phenolic compound content in the sample (Putri, Karim, Khairiyah, & Ramadhani, 2023). Next, 4 mL of saturated Na_2CO_3 solution was added and vortexed for 1 minute. Phenolics are only found in basic solutions, so Na_2CO_3 is added to form an alkaline atmosphere so that the Follin-Ciocalteu reduction reaction occurs by the hydroxyl groups of the phenolics in the sample.

Determination of total phenol content in tea leaves using gallic acid standards. Gallic acid is a constituent of phenolic compounds, which have a hydroxyl group and a conjugated double bond on each benzene ring, so that when it reacts with Follin Ciocalteu's reagent, it forms a more complex compound, it is used as a standard in determining total phenol (Putri et al., 2023). A stock solution of 1000 ppm gallic acid and standard solutions of 12.5 ppm gallic acid, 15 ppm, 17.5 ppm, 20 ppm, and 22.5 ppm were prepared. 1 mL of each standard solution was pipetted and added with 0.5 mL of Follin Ciocalteu reagent. Leave it for 8 minutes. Add 4 mL of saturated Na_2CO_3 solution and vortex for 1 minute.

E. The Gallic acid determination

Determination of gallic acid content using a UV-Vis spectrophotometer was carried out at a wavelength of 794 nm (Kusmiyati et al., 2016). Measurements were carried out on standard solutions to obtain concentration and absorbance data, which were plotted into a standard curve.

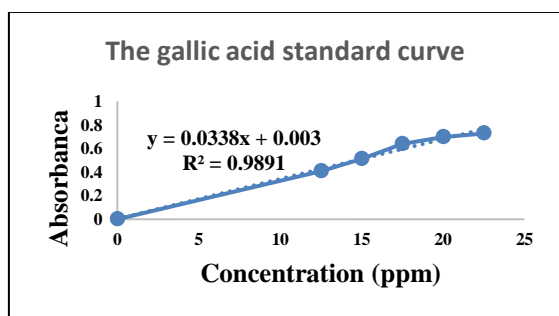


Figure 4. The gallic acid standard curve

Based on the standard curve, the equation obtained $y = 0.0338x + 0.003$ with $R^2 = 0.9891$. This equation is

linear, so it can be used to determine the total phenol content in the sample. From the equation $y = 0.0338x + 0.003$, the total phenol concentration in the sample can be calculated based on the sample absorbance results. The total phenol concentration in the sample was obtained at 1.7071 ppm. Next, the total phenol content was calculated (%b/b), and the results were 34.142%. The highest concentration of the secondary metabolite in tea leaves is the phenol group (Anjarsari, 2016). Based on research conducted by Danniells in 2008, green tea contains 30-40% polyphenols, while black tea only has 3-10%. From this research, the total phenol content is obtained about 34.142%. This number is in the range of green tea polyphenol content. Tea leaves that have not undergone a fermentation process and have been steamed to deactivate the polyphenol oxidase enzyme make the phenol levels in this study match the polyphenol levels of green tea.

CONCLUSION

Based on the results of research on tea leaves from Sirah Kencong Blitar Plantation, the caffeine, tannin, and total phenol levels produced are in the range of green tea caffeine levels, namely caffeine of 1.649%, tannin of 7.095% and total phenol of 34.142%.

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