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Tannins inhibition of tea leaves (*Camellia sinensis*) against *Escherichia coli* diarrhea-causing bacteria

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Abstract. *Cahyani RK, Susilowati A, Sari SLA. 2019. Tannins inhibition of tea leaves (*Camellia sinensis*) against* Escherichia coli *diarrhea-causing bacteria. Bioteknologi 16: 48-52.* Diarrhea is often suffered by people and has become the biggest cause of death in developing countries. Diarrhea can be caused by *Escherichia coli* infection. Treatment with antibiotics can lead to resistance, so herbs like tea leaves can be used as an alternative to overcome diarrhea. This research aims to determine the effect of tannins in ethanol extract of tea leaves from Kemuning Estate against diarrhea-causing bacteria. Tea leaves powder was macerated using ethanol 70% to obtain a paste extract for an antibacterial test. The antibacterial test was conducted using contact bioautography methods, which combine thin-layer chromatography with the response of the bacteria test by antibacterial activity. The test results showed that the antibacterial activity of tannins in ethanol extract of tea leaves with a concentration of 30% and 50% could inhibit the growth of *E. coli*. The strongest concentration to inhibit the *E. coli* bacteria is 50% which contains 13.8% of tannins and has a 14 mm inhibition zone diameter. In comparison, a concentration of 30%, which contains 8.28% of tannins and an inhibition zone diameter of 10 mm, is included in moderate categorized inhibition. Tannins analysis by Thin Layer Chromatography (TLC) using $FeCl_3$ reagent formed blue ink or black colors, which means tannins in tea leaves are classified as the hydrolyzed tannins. Analysis of quality tannins with the Folin-Ciocalteu method showed that the tannins content in ethanol extract of tea leaves from Kemuning Estate was 27.6%.

Keywords: Bioautography, diarrhea, *Escherichia coli*, *Camellia sinensis*, tannin

INTRODUCTION

Diarrhea is derived from the Greek and Latin words dia, which means to pass, and rhein, which means to flow. Diarrhea occurs when stools become much watery in consistency and occur more frequently than usual (Nuratmi et al. 2006). Diarrhea is a very prevalent disease in children and adults alike. Until now, diarrhea has been one of the leading causes of death in children under five years. According to Alexander et al. (2013), diarrhea is responsible for 15% of child mortality. Adults in Indonesia experience over 100 million episodes of diarrhea per year. Simultaneously, the WHO estimates that over 4 billion cases of diarrhea occur each year, with a mortality rate of 3-4 million (Zein et al. 2004). Diarrhea is caused by contaminated beverages and rotten food contaminated with bacteria, fungus, or poisonous or harmful microorganisms. Diarrhea can be caused by germs such as *Escherichia coli*. Enteropathogenic bacteria produce diarrhea by attaching to epithelial cells with or without mucosal injury, invading the mucosa, and producing enterotoxins or cytotoxins.

If antibiotics such as ciprofloxacin, chloramphenicol, or tetracycline are taken continuously, resistance will develop. Bacterial resistance occurs as a result of a mutation in a bacterial gene. According to Ameri (2014), the proliferation of antibiotic resistance genes among different disease-causing bacteria has increased, lowering an antibiotic's effectiveness.

The importance of traditional medicine is becoming more apparent now, as is the growing understanding of the usefulness of numerous medicinal plants in Indonesia. Antidiarrheal properties of medicinal plants such as tea leaves are well established. Traditionally, tea has been used to help alleviate or even stop diarrhea. Tea is an indigenous plant to Indonesia and has a long history of widespread distribution. Tea cultivation is concentrated in the Surakarta area, specifically at the Kemuning tea estate in the Ngargoyoso District, Karanganyar, Indonesia. This plantation's tea has been processed and marketed domestically and internationally. Tea is a versatile plant utilized in various ways, including as an ingredient in refreshing beverages and a component of pharmaceutical and cosmetic products. The health advantages of tea are derived from the presence of key components such as caffeine, tannins, essential oils, vitamins, and minerals (Loto 2011). Tannins have an astringent flavor and are phenolic, which means they can be employed as antibacterial agents, antioxidants, and tumor inhibitors (Vitanti et al., 2012; Mailoa et al., 2014). Tannins are considered to be antibacterial due to their ability to build complex compounds with proteins via hydrogen bonds; when tannins and proteins establish hydrogen bonds, protein denaturation interferes with bacterial metabolism.

According to Sung et al. (2012), tannins are categorized into two types according to their molecular structure: condense tannins (catechins) and hydrolyzed tannins (gallic acid). Hydrolyzed tannins have the ability to contract bigger tissues in response to irritation-induced diarrhea. The broken tannate protein will attach to hydrolyzed tannins that pass through the colon, reducing small intestinal secretion and constipation. Tannin condensed has a protective function. Tannins can be used as diarrhea medications because they help to minimize fluid loss from the gastrointestinal tract and restore the intestinal flora's equilibrium (Defrin et al. 2010). Tea leaf extract has been shown in earlier research to prevent the growth of *E. coli* bacteria, which is one of the bacteria that causes diarrhea. According to Widiana (2012), tea leaf extract has a minimum inhibitory concentration of 3.125% against *E. coli* bacteria. However, it is unknown which individual chemicals in tea leaves can suppress *E. coli* bacteria based on the results of these investigations. As a result, this study focused on the unique properties of tea leaf extract, specifically tannins, which limit the growth of diarrhea-causing bacteria such as *E. coli*. The tannins from tea leaves were studied for their ability to prevent the growth of *E. coli* bacteria. Tea leaves contain tannins, which may be used as an alternative diarrhea medication.

MATERIALS AND METHODS

Material

The materials used were tea leaves (*Camellia sinensis* (L.) Kuntze) from the Kemuning plantation, Karanganyar, Indonesia, isolates of *E. coli* bacteria obtained from diarrhea patients and cultured in the Microbiology Laboratory of the Faculty of Medicine, Airlangga University Surabaya, nutrient broth (NB), nutrient agar (NA), gallic acid, Folin-ciocalteu, Na_2CO_3 15%, acetic acid, butanol, aquadest, $FeCl_3$, and 70% ethanol.

Method

Ethanolic extraction of tea leaf

To make a thick paste for antibacterial testing, the tea leaf powder was macerated with 70 % ethanol for 3 x 24 hours with regular stirring (Harborne 1996), then filtered through Whatman filter paper no.42 and evaporated in a rotary evaporator at 50°C.

Analysis of tannin content

Analysis of tannin content was based on the Folin-ciocalteu method with a gallic acid standard. Standard solutions of 0, 10, 25, 50, 100, and 250 ppm gallic acid were taken as much as 2 mL and added 0.2 mL of Folin-Ciocalteu reagent and 1 mL of 15% Na_2CO_3 solution, then shaken until smooth. The solution was allowed to stand for 2 hours at room temperature, and its absorbance was measured using a UV-Vis spectrophotometer of 765 nm. For the sample analysis, 100 mg of tea leaf extract was taken, then 0.2 mL of Folin-Ciocalteu reagent was added, 1 mL of 15% Na_2CO_3, and 2 mL of distilled water were shaken until smooth. The solution was allowed to stand for 2 hours at room temperature, and its absorbance was measured using a UV-Vis spectrophotometer of 765 nm (Yulia 2006).

Thin-layer chromatography (TLC) analysis

TLC analysis of tannin compounds in tea leaf extract with concentrations of 10%, 30%, and 50% used silica gel GF254 as a stationary phase and a mobile phase in the form of butanol-acetic acid-water (2:0,5:1,1 v/v). Spot stain detection was carried out using UV254 and UV365 light and an Rf value of 0.22-0.92 (Yamuna et al. 2012). Qualitative analysis of tannins was carried out by detecting 1% $FeCl_3$ spray (Chavan and Amarowicz 2013).

Antibacterial activity test with bioautography method

Researchers used the contact bioautography approach to determine antibacterial activity. The test bacteria were cultured in a petri dish using a 10 mL sterile NA medium using the swab method. Following drying, the TLC plate was mounted to the surface of the NA medium for 20-30 minutes with a 10%, 30%, or 50% extract solution. After removing the TLC plate, the petri dish was incubated for 24 hours at 37°C (Akhyar 2010). The inhibitory zone was seen by checking for a bright area that was not overrun by bacteria. A caliper or ruler is used to measure the diameter of the inhibitory zone vertically, horizontally, and diagonally and then averaged in millimeters (Dharmawati 2011). The positive control plate included 13.8% gallic acid, while the negative control plate contained butanol-acetic acid-water.

RESULTS AND DISCUSSION

The tannin content of tea leaf extract

Folin-Ciocalteu reagent and gallic acid standard were used to determine the tannin content. The standard gallic acid curve (Figure 1) was designed to determine the regression equation for determining the tannin content in tea leaf extract via phenolic analysis (Sultana et al., 2012).

According to the regression equation derived from the standard curve, the tannin concentration in tea leaf extract was 27.6%, implying 0.276 grams of tannin in 1 gram of tea leaf extract. Table 1 shows the tannin content of various concentrations of ethanol extract from tea leaves.

Table 1. The concentration of tea leaf ethanol extract and tannin content

Tea leaf ethanol extract concentration (%)	Tannin content (%)
10	2.76
30	8.28
50	13.8

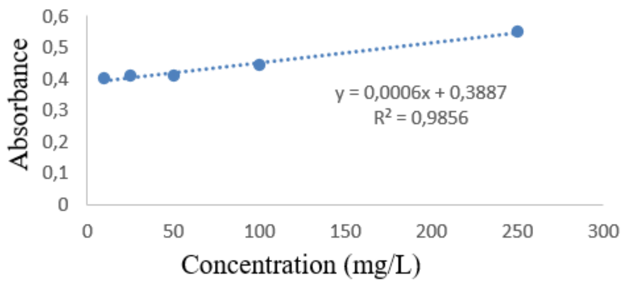

Figure 1. Gallic acid standard curve

The tannin content of the tea leaves obtained from the Kemuning Plantation was 27.6%, while the tannin content obtained from the Nusantara VIII Plantation, Sukabumi, was 0.13%. The Kemuning Plantation produced tea leaves with a tannin content of 27.6%, while the Nusantara VIII Plantation produced tea leaves with a tannin content of 0.13% (Yulia 2006). The tannin concentration in tea leaves from Kemuning Plantation is quite high, demonstrating that the plantation has a high tannin content.

TLC tannins tea leaf extract

Tea leaf extract was subjected to TLC to separate tannin components. This method is used to separate one substance from another. It is based on the difference in distribution between two phases, which are the stationary phase and the mobile phase. Silica gel GF254 is used as the stationary phase to carry out this TLC procedure. The solvent is butanol: acetic acid: water eluent in the ratio of 2:0.5:1.1 (v/v), with a swelling distance of 5 cm between the stationary phases. However, according to Hayati et al. (2010), the butanol: acetic acid: water (BAA) mixture is the most effective eluent for separating tannin compounds because the eluent composition is particularly polar, allowing it to separate tannin compounds that are also polar.

TLC chromatogram of tea leaf ethanol extract at a concentration of 50% (Figure 2) showed there were 3 separation spots with Rf values of 0.22, 0.72, and 0.92. Of the three stains formed, the second stain was suspected to be a hydrolyzed tannin compound or error, which had an Rf value of 0.72, and the color of the stain, when irradiated with UV254 light, became violet. In contrast, UV365 light produced two colors: purple and reddish-purple. This result is reinforced by Harborne (1996) that UV254 can detect tannins in the form of purple stains, and according to Hayati et al. (2010), color chromatogram stains with UV365 light produced two colors at different Rf, namely greenish-brown and reddish-purple. The eluted ethanol extract of tea leaves, when sprayed with 1% $FeCl_3$, becomes ink blue or black, indicating the presence of hydrolyzed tannins (Sa'adah 2010).

Gallic acid as a positive control only formed 1 spot stain with an Rf value of 0.72 (Figure 3).

Antibacterial activity in the bioautographic method

Contact bioautography is used to determine the antibacterial activity of tannin components found in the tea leaf extract. This method was chosen due to the clarity of the data obtained without using methyl thiazole tetrazolium (MTT). The tea leaf ethanol extract concentrations utilized to determine the bioautography method's antibacterial efficacy were 10%, 30%, and 50%. Figure 4 illustrates the results of the bioautography test.

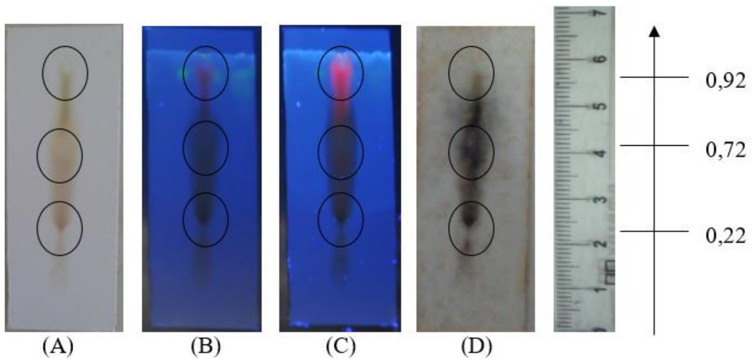

Figure 2. TLC chromatogram of tea leaf ethanol extract detection by (A) visible light, (B) UV254 light, (C) UV365 light and (D) 1% $FeCl_3$ spray detection

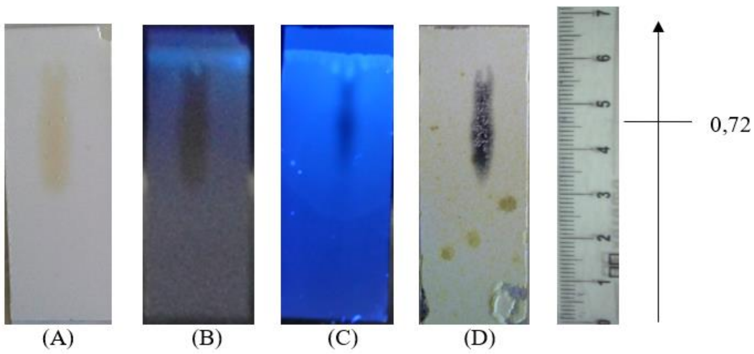

Figure 3. TLC chromatogram of gallic acid 13.8% detection by (A) visible light, (B) UV254 light, (C) UV365 light and (D) 1% $FeCl_3$ spray detection

The results indicated that the tannin compound from the ethanol extract of tea leaves had a maximum inhibition zone diameter of 14 mm at a 50% concentration. The 30% concentration had an inhibition zone diameter of 10 mm (Table 2) as a comparison. However, the tannin component from a 10% ethanol extract of tea leaves did not generate an inhibitory zone. This condition is most likely because the concentration of tannin compounds is so low. According to Ristiningsih (2009), the concentration increases the size of the inhibitory zone. The active ingredients in it are becoming more concentrated, increasing the amounts of bacteria inhibited.

As a positive control, gallic acid (13.8%) exhibited a bigger and more distinct inhibitory zone than the tea leaf tannin complex. It is most likely because gallic acid is a pure chemical, but the tannins in tea leaf ethanol extract are more complicated. The inhibitory zone was not generated when butanol: acetic acid: water was used as a negative control. It demonstrates that the TLC mobile phase does not affect the suppression of *E. coli* bacterial growth.

The study's results indicated that the tannin components in the ethanol extract of tea leave possessed moderate to strong inhibitory activity against the *E. coli* bacteria that cause diarrhea. According to Davis and Stout in Nurrakhman (2011), the provisions for the strength of antibacterial power are as follows: an inhibition area of 20 mm or greater is considered very strong, an inhibition area of 10-20 mm is considered strong, an inhibition area of 5-10 mm is considered medium, and an inhibition area of 5 mm or less is considered weak.

According to Mailoa et al. (2014), tannins exert antibacterial action by entering the bacterial cell wall. Gram-negative bacteria have thinner cell walls, allowing tannins to more easily destroy the proteins found in the cell walls of *E. coli* bacteria. Because protein is a component of bacteria's cell walls and plasma membranes, substances that induce bacterial metabolic problems can easily enter proteins in damaged or denatured cell walls. Tannins can generate hydrogen bonds with proteins found in bacterial cells; when these hydrogen bonds form, protein denaturation occurs, interfering with bacterial metabolism. Tannins are hypothesized to limit the growth of *E. coli* bacteria based on this interaction. Tannins can also impede the growth of germs and destroy them via interactions with cell membranes. Tannins react with proteins in cell membranes to produce hydrogen bonds, denaturing the proteins. Additionally, tannins can react with phospholipids, damaging cell membranes and allowing for the leaking of compounds that inactivate bacterial enzyme systems. Damage to cell membranes prevents the admission of food items or nutrients required for bacteria to make energy, inhibiting bacterial growth and perhaps cell death.

Table 2. The concentration of ethanol extract in tea leaves, along with their tannin content (grams) and the diameter of the inhibition zone (mm) for the growth of *E. coli* bacteria

Test sample	Tannin content (g)	Inhibition zone diameter (mm)
Tea leaf ethanol extract 10%	0.0276	-
Tea leaf ethanol extract 30%	0.0828	10
Tea leaf ethanol extract 50%	0.138	14
Positive control	0.138	20
Negative control	0	-

Note: Positive control: gallic acid 13.8%, Negative control: mobile phase (butanol-acetic acid-water)

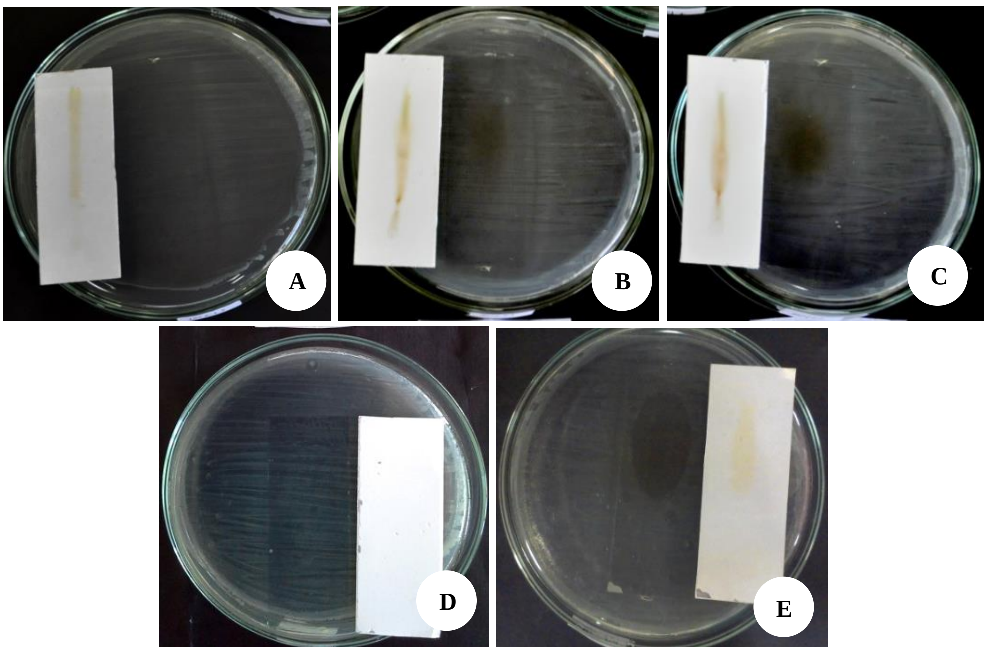

Figure 4. Antibacterial inhibition zone of tea leaf ethanol extract with concentration (A) 10%, (B) 30%, (C) 50%, (D) negative control (butanol: acetic acid: water) and (E) control positive (gallic acid 13.8%)

In comparison to the tannin extract of akway leaves (*Drimys Piperita* Hook.f.), the tannin extract of tea leaves (*C. sinensis*) has greater potential as antibacterial inducing diarrhea. According to Situmorang (2009), akway leaf tannin extract with a 12.62% tannin content inhibits *E. coli* bacteria with a 3.93 mm clear zone diameter. In this investigation, the tannin extract of tea leaves with a tannin level of 13.8 % inhibited *E. coli* bacteria with a clear zone diameter of 14 mm.

To conclude, Tannin compounds in the ethanol extract of tea leaves from the Kemuning plantation have antibacterial activity against *E. coli* bacteria that cause diarrhea. The tannin content in the ethanol extract of tea leaves from the Kemuning plantation is 27.6%. The strongest inhibitory concentration of tannin compounds in the ethanolic extract of tea leaves that could inhibit *E. coli* bacteria was 50%, with a tannin content of 13.8% and an inhibition zone diameter of 14 mm.

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