DOI: http://dx.doi.org/10.33846/hn80103
http://heanoti.com/index.php/hn

RESEARCH ARTICLE
URL of this article: http://heanoti.com/index.php/hn/article/view/hn80103

Antimicrobial Activity of Green Tea Extract Against of *Escherichia coli*

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ABSTRACT

Research into substances that are efficacious as new antibiotics to inhibit or kill bacteria that are resistant to antibiotics needs to be done. One simple that has antibacterial effects is green tea leaves *(Camellia sinensis).* The anti-Sacher content of green tea leaf anchovies (*Camellia sinensis),* namely alkaloids, saponins, tannins, and catechins (polyphenols). Tea is one of the superior products of plantations in Indonesia. The purpose of this study was to determine the optimization of the inhibitory power of green tea leaf extract *(Camellia sinensis)* against the growth of *Escherichia coli* bacteria. This research was experimental laboratory using the *Kirby Bauer disk diffusion* method, namely *Mueller Hinton* Agar (MHA) media that has been planted with cultures of *Escherichia* coli women given *disks* soaked in green tea leaf extract *(Camellia sinensis)* concentrations of 25%, 50%, 75%, and 100% and then incubated for 24 hours. From the results of the study, it can be concluded that green tea leaf extract *(Camellia sinensis)* can be used as an antibacterial. The optimal results in this study are *Escherichia coli* bacteria at a concentration of 25% with an average diameter of 10.25 mm. This is because the main content of green tea leaves *(Camellia sinensis)* catechins and alkaloids as powerful antibacterials

Keywords: green tea; inhibitory; *Escherichia coli*

INTRODUCTION

Microorganisms are found anywhere, in water, air, soil, and living things including human body tissues.(1) One type of microorganism that has a very small size is bacteria.(2) Some bacteria can cause disease in humans.(3)

Diarrhea can be caused by microorganisms, one of which is *Escherichia coli* bacteria which is a normal flora in the digestive tract but has the potential to cause pathogenic diseases if the number of *Escherichia coli* in the gastrointestinal tract increases.(4)

The infection can be treated using antibiotics.(5) Antibiotics can cure diseases caused by bacterial infections, and irrational use of antibiotics can increase bacterial resistance to antibiotics.(6) Researchon substances that are efficacious as new antibiotics to inhibit or kill bacteria that are resistant to antibiotics needs to be done. One way is to use plants that can kill bacteria to avoid resistance. One simplisia that has antimicrobial effects is green tea leaves *(Camellia sinensis).* Some of the chemical ingredients possessed by green tea that act as antibiotics are alkaloids, saponins, tannins, catechins (polyphenols).(7)

Based onthe description above, it is necessary to conduct an examination to prove that whether green tea leaf extract *(Camellia sinensis)* is also able to inhibit the growth of *Escherichia coli* bacteria by its use as an antibacterial.

METHODS

This type of research conducted in experimental laboratory which aims to determine the effect of giving green tea leaf extract *(Camellia sinensis)* on the growth of *Escherichia coli* bacteria. The test materials used were green tea leaves *(Camellia sinensis)* obtained from the Sukun area, Malang, East Java, and *Escherichia coli* ATCC 25922 bacteria obtained from *Balai Besar Surabaya* Health Laboratory.

Some of the tools and media to be used in this study were previously sterilized using an *autoclave* at 121ºC for 15 minutes. Weighing *Mueller Hinton Agar* (MHA) media powder 17.5 gr dissolved in 100 mL equates to an erlenmeyer flask and is covered with fat cotton. The solution is heated until the powder is completely soluble and

homogeneous but not to boiling, then the pH is measured to pH 7.3 ± 1 . Sterilized on an autoclave at a temperature of 1210C for 15 minutes. After sterilization, wait until lukewarm (± 40°C). The media can be poured aseptically at 10-20 mL Petri dish, then left at room temperature until the media solidifies completely.

Suspends pure cultures of *Escherichia coli* bacteria into 9 mL PZ 0.9% sterile. Then it is equivalent to the Mc Farland standard of 0.5 (0.5 mL BaCl 2 1.175% + 99.5 mL H2SO4 1%). If it is cloudy, it can be added PZ 0.9% sterile, and if it is clear, it can be added to a suspension of bacteria until the turbidity is comparable to the turbidity of McFarland 0.5. The bacterial suspension was then made 10:13 thinning with sterile 0.9% PZ as diluent. A suspension of *Escherichia coli* bacteria of 10 13 was grown on *Mueller Hinton Agar* (MHA) media.

Samples of green tea leaves *(Camellia sinensis)* were made simplistic, then maceration method extraction was carried out with 96% ethanol solvent and the solvent was replaced every 24 hours maceration was done by soaking Simplisia with the appropriate solvent in a tightly closed container at room temperature, the result is a viscous extract, green in color and distinctive smell so that the the concentration of the extract is obtained from Green tea leaf ethanol *(Camellia sinensis)* and then made in several different concentrations. concentration 25% (0.25 mL green tea leaf extract + 0.75 mL sterile equates), concentration 50% (0.5 mL green tea leaf extract + 0.5 mL sterile equates), concentration 75 % (0.75 mL green tea leaf extract + 0.25 mL sterile equates), 100% concentration (1 mL of green tea leaf extract).

The disc used is a ready-to-use disc with a diameter of 6 mm manufactured by Macherey-Nagel, Germany. The disc was immersed for 15-30 minutes into the extract of green tea leaves (*Camellia sinensis*) at concentrations of 25%, 50%, 75%, 100 %, positive control (*Ciprofloxacin*) and negative control (Aquadest sterile). After the bacteria are aseptically inoculated on MHA media, place the disc on the media using sterile tweezers. Incubation for 1x24 hours at a temperature of 37ºC, observing and measuring the diameter of the inhibitory zone formed.

RESULTS

At a concentration of 25%, there was an inhibitory zone with a diameter of 20.00 mm, at a concentration of 50% there is an inhibitory zone with a diameter of 21.00 mm, at a concentration of 75% there is an inhibitory the zone that diameter of 19.00 mm, at a concentration of 100% there is an inhibitory zone g diameter of 22.00 mm, in positive control using *Ciprofloxacin* there is an inhibitory zone with a diameter of 49.00 mm, And in the negative control using sterile aquades, there is no inhibition zone.

Table 1. Bacterial inhibitoion test of green tea leaf extract *(Camellia sinensis*) against the growth of *Escherichia coli* bacteria

No.	Replication	Green tea leaf extract concentration					
		25%	50%	75%	100%	Positive control	Negative control
1	I	12.00	7.00	0.00	0.00	44.00	0.00
2	II	11.00	6.00	0.00	0.00	46.00	0.00
3	III	9.00	7.00	0.00	0.00	50.00	0.00
4	IV	9.00	6.00	0.00	0.00	44.00	0.00
∑		41.00	26.00	0.00	0.00	184.00	0.00
Average diameter (mm)		10.25	6.50	0.00	0.00	46.00	0.00

Based on the results of the study, the optimum inhibitory power obtained by *Escherichia coli* bacteria at a concentration of 25% has an inhibitory zone with an average diameter of 10.25 mm.

These results on *Escherichia coli* bacteria show that the value obtained was less than 0.05, so data on each concentration of tea leaf extract green *(Camellia sinensis)* was not normally distributed. From these results *Escherichia coli* bacteria had a p-value of 0.000, then the data was not homogeneous. The data continued using a non-parametric test: *Kruskal Wallis.* These results on *Escherichia coli* bacteria showed p-value of 0.001 which showed the existence of an optimal inhibitory zone in the administration of green tea leaf extract *(Camellia sinensis)* to plant pests *Escherichia coli* bacteria.

DISCUSSION

Test optimization of the inhibitory power of green tea leaf extract (*Camellia sinensis*) against the growth of *Escherichia coli* bacteria using the diffusion method was carried out to determine the antibacterial power of green tea leaf extract (*Camellia sinensis*) against the growth of *Escherichia coli* bacteria. At the research stage, tests were carried out using the diffusion method which was carried out by immersion of discs in green tea leaf extracts with concentrations of 25%, 50%, 75%, and 100%, then the suspension of *Escherichia coli* bacteria was inoculated on *Mueller Hinton Agar* (MHA) media and then incubated for 24 hours with a temperature of 37 °C then observed an inhibitory zone marked by a clear zone that occurs so that it can be calculated using a caliper.

Based on the results of antibacterial test research of green tea leaf extract *(Camellia sinensis)* against the growth of *Escherichia coli* bacteria from 4 concentrations, namely 25%, 50%, 75%, and 100% with 4 times replication using diluent sterile aqua distillate. Sterile aqua distillate does not have antifungal properties so it can also be used as a negative control, while for positive controls use *Ciprofloxacin*. *Ciprofloxacin* is a *fluoroquinolone class antibiotic, Ciprofloxacin* is effective for therapy and does not cause resistance.(8)

The optimum inhibition zone of *Escherichia coli* bacteria against green tea leaf extract *(Camellia sinensis)* is found at a concentration of 25% having a diameter of 10.25 mm, it can be known that at an average concentration of 50% 6.50 mm in diameter and at concentrations of 75% and 100% do not produce inhibitory zones. It can be concluded that the higher the concentration, the smaller the resulting diameter.

This can occur by several factors, namely, *Escherichia coli* is one of the Gram-negative bacteria that have a cell wall coated with an outer membrane containing proteins, phospholipids, and lipopolysaccharides.(9)

The outer wall of *Escherichia coli* bacteria has high permeability properties so that the active substances in green tea extract cannot be maximally introduced into the bacterial cells resulting in less than optimal extracts in inhibiting bacterial growth.(10)

The bacterial wall also consists of lipoproteins containing protein molecules namely porins and lipopolysaccharides.(11) These porins are hydrophilic, while extracts are hydrophobic.(12) Because of this difference in properties, the component molecules of the extract become more difficult to enter the bacteria.(13) In addition, the outer wall of *Escherichia coli* bacteria contains many nonpolar lipid layers, while extracts are polar.(14) The existence of differences in these properties causes the extract component molecules to also become more difficult to enter the bacteria.(15) Therefore, this can affect the working activity of green tea ethanol extract in inhibiting the growth of Escherichia coli. Tannins also play an important role as antibacterial because they can convert bacterial proteins into complex compounds through hydrogen compounds, disrupting the stability of the bacterial cell wall Furthermore, it decreases the selective permeability function of the membrane, decreases the active transport system, and disrupts the bacterial cell structure.(16) Another tannin reaction can bind peptidoglycan to the bacterial membrane.(17)

Phenol derivatives can interact with bacterial cells through an adsorption process involving hydrogen bonds, resulting in bacteria-denatured cell proteins and damaged membranes Cells will thus result in the denaturation of cell proteins.(18) Damage to the cell membrane can inhibit the entry of substances in the cell such as organic ions, enzymes, and amino acids can exit the cell.(19) This ATP produced will decrease and cause stunted bacterial growth and cell death.(20) Green tea extract has anti-microbial activity against *Escherichia coli*.

CONCLUSION

From the results of the study Test Optimization of the Inhibitory Power of Green Tea Leaf Extract *(Camellia sinensis)* Against the Growth of *Escherichia coli* Bacteria. Zona optimal inhibition of green tea leaf extract *(Camellia sinensis)* against the growth of *Escherichia coli* bacteria at a concentration of 25% with an average diameter of 10.25 mm. Tests on green tea leaf extract (*Camellia sinensis*) on *Escherichia coli* bacteria with concentrations of 25%, 50%, 75%, and 100% obtained an average diameter of 10.25; 6.50; 0.00; 0.00. So that the greater the concentration of green tea leaf extract solution, the smaller the diameter of the resulting inhibitory zone.

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