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RESEARCH ARTICLE

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Phytochemical Screening and In Vitro Antibacterial Activity of Green Tea (*Camellia Sinensis* L) Extract Against *Staphylococcus Epidermidis*

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ABSTRACT

This study aimed to determine phytochemical screening and ethanol extract activity of the green leaves against *Staphylococcus epidermidis*. The microbial activity of the ethanol extract was carried out using the diffusion and dilution method with concentrations used of 1.5%, 2.5%, 3.5%, 4.5%, and 5.5% accompanied by three repetitions. The results showed that the ethanol extract of green tea leaves contained alkaloids, saponins, steroids or triterpenoids, flavonoids, polyphenols, and tannins and had the largest inhibition zone diameter of 30mm at a concentration of 4.5%. KHM (Minimum Inhibitory Content) at a level of 4.5% and KBM (Minimum Kill Rate) at a concentration of 5.5%. Analysis of the data with the normality test then proceed with the Kruskal Wallis test obtained a value of $P = 0.008$. With the value $P < \alpha$, where $\alpha = 0.05$, which means significant. So it can be concluded that the ethanol extract of green tea leaves (*Camellia sinensis* L) effectively inhibits the growth of *Staphylococcus epidermidis* bacteria.

Keywords: Phytochemical, *Staphylococcus epidermidis*, green tea

INTRODUCTION

Background

Acne is a skin problem commonly known as acne vulgaris, a condition where the skin's pores are blocked. Some of the bacteria that cause acne to grow are *Staphylococcus epidermidis*⁽¹⁾. In general, Indonesians prefer antibiotics to deal with infections. Antibiotics can be produced by semi-synthetic and synthetic methods to treat and prevent diseases caused by bacteria. Medicines manufactured from chemical synthesis are indeed effective in operating conditions, but on the other hand, can also have harmful side effects. Antibiotic resistance in the treatment of acne is increasing in various countries, which will have an impact on the ineffectiveness of antibiotics used⁽²⁾.

Antibiotics can be developed through the exploration of new products based on natural ingredients. Natural substances have secondary metabolites or other potential antibacterial compounds. Besides, the use of traditional elements also shows smaller side effects than the use of synthetic drugs.

Some studies report that herbal ingredients can inhibit the growth of *Staphylococcus epidermidis* bacteria. One of the herbal ingredients used as a treatment is green tea. Parts of tea leaves that contain antibacterial power are phenol or polyphenol substances (catechins, tannins, flavonoids) and non-phenol substances (alkaloids and flour) that can inhibit and kill bacteria⁽³⁾.

Catechin compounds are polyphenol compounds present in green tea that are antimicrobial (bacteria and viruses), antioxidants, antiradiation, strengthen blood vessels, launch urine secretion and inhibit the growth of cancer cells⁽⁴⁾. Various active compounds contained in green tea indicate that green tea has excellent

antibacterial effectiveness. Based on the description above, the researcher wants to examine the efficacy of ethanol extract of green tea leaves (*Camellia sinensis* L) on the growth of *Staphylococcus epidermidis* bacteria in vitro.

METHODS

Plant Material

Leaves of green tea (*Camellia sinensis* L) were collected from Batu, Malang and identified by the taxonomist of the UPT Laboratorium Herbal Materia Medica Batu

Extraction Process

Leaves of the plant were sun-dried and then crushed into small pieces. Then 100 g of dried leaf powder was taken in a separate container. To this, 500 ml of ethanol was added and kept for 24h at room temperature with periodic shaking and then filtered with Whatman No 1. Filter paper and the filtrate were collected. The procedure was repeated three times with a new volume of ethanol. The filtrates were pooled to ensure maximum extraction. Then the extract was dried and weighed. The yield was 38.4822 g

Phytochemical Screening

The ethanol extract of green tea leaves was then subjected to phytochemical screening to determine the content of alkaloids, saponins, steroids/triterpenoids, flavonoids, polyphenols and tannins, anthraquinones. These were identified by characteristic color changes using standard procedures^(5,6,7)

Equipment Sterilizers

The equipment that will be sterilized is covered with fatty cotton and wrapped, then sterilized at 121°C for 15 minutes.

Dilution Method

Green tea leaf extracts with concentrations of 1.5%, 2.5%, 3.5%, 4.5%, and 5.5% are put into a test tube that has been labeled. MHB (Muller Hinton Broth) pipettes mixed with a bacterial suspension of 0.5 ml in each test tube. Next, pipette each concentration of green tea extract as much as 0.5 ml and put it into a test tube filled with MHB (Muller Hinton Broth) media and bacterial suspension then homogeneous. After that, it was incubated at 37°C for 18-24 hours, and turbidity was seen.

Diffusion Method

Blank disks were prepared, and these disks were dripped 20µl each concentration of green tea leaf extract until all the liquid seeped into the disk discs. Each disc saturated with green tea extract concentration was then placed on the surface of the MHA media that had been etched with suspension bacteria and slightly pressed with tweezers until it adhered perfectly. Erythromycin antibiotic discs that function as positive controls and disk discs that have been saturated with sterile aqua dest as negative controls are affixed to the MHA media. Adjust the distance of the disc (15 mm between the other drives and the discs that have been attached to the media's surface must not be moved or moved). Media that has been planted with discs is incubated in an incubator for 24 hours in an upside-down position at 37 ° C. After incubation, the clear zone formed will be measured by the bar and converted to mm (millimeters).

RESULTS

Phytochemical Screening

Green tea (*Camellia sinensis* L) was evaluated qualitatively for the presence and absence of different secondary metabolites by applying devised biochemical test for alkaloid, saponin, steroide/triterpenoid, flavonoid, polyphenol and tannin, antraquinon. The results are arranged in table 1. alkaloid, saponin, steroide/triterpenoid, flavonoid, polyphenol and tannin were found in leaves of green tea (*Camellia sinensis* L). Antraquinon were absent in this plant part.

Table 1. Phytochemical screening of Extract Ethanol Green tea leaves (*Camellia sinensis* L)

Phytochemical	Method	Result
Alkaloid	Mayer	+
	Wagner	+
Saponin	Foam test	+
Steroid/Triterpenoid	Lieberman-Burchard	+
	Salkowski	+
Flavonoid	Batesmith and metcalf	+
Polifenol and tannin	Gelatin	+
	Ferriklorida	+
Antraquinone	Borntrager	-

Positive (+) = present and negative (-) = absent

Antibacterial Activity

Difussion Method

Based on research on testing the effectiveness of ethanol extracts of green tea leaves (*Camellia sinensis* L) on the growth of *Staphylococcus epidermidis* bacteria in vitro using the diffusion method. Then get the following results:

Table 2. Zone of inhibition by extract ethanol green tea leaves (*Camellia sinensis* L) with diffusion method

Concentration of extract	Inhibitory Zone Diameter (mm)			Average
	I	II	III	
1.50%	23	27	27	26
2.50%	25	25	25	25
3.50%	29	29	25	28
4.50 %	30	30	30	30
5.50%	0	0	0	0
Positive control	29	29	29	29
Negative control	0	0	0	0

Positive control = Erythromicin, Negative control = Aquadest

Because the data is typically distributed and not homogeneous, a Kruskal Wallis test is performed and based on the results of the Kruskal Walis test the P Asymp Sig (2-tailed) value of $0.009 \leq \alpha$ (0.05) can be concluded that H_0 is rejected, and H_1 is accepted which can be interpreted that in this research data there are significant differences in inhibition zones of Green Tea Leaf Extract (*Camellia sinensis* L) at various concentrations of *Staphylococcus epidermidis*.

Dilution Method

Based on research on testing the effectiveness of ethanol extracts of green tea leaves (*Camellia sinensis* L) on the growth of *Staphylococcus epidermidis* bacteria in vitro using the dilution method. Then get the following results:

Table 3. Zone of inhibition by extract ethanol green tea leaves (*Camellia sinensis* L) with dilusion method

Concentration of extract	Turbidity of extract after 37°C incubation for 24 hours	Colony growth on MHA media after incubation 37°C for 24 hours	The inhibitory ability of green tea extract against <i>Staphylococcus epidermidis</i> bacteria
1.50%	+	+	-
2.50%	+	+	-
3.50%	+	+	-
4.50 %	+	+	-
5.50%	-	-	+
Positive control	-	-	+
Negative control	+	+	+

The concentration of green tea leaf extract (*Camellia sinensis* L) is the concentration of the material tested against *Staphylococcus epidermidis* bacteria; Turbidity of the extract after 37°C incubation for 24 hours (Positive (+): turbidity indicates bacterial growth, Negative (-): no turbidity (clear) indicates inhibition of bacteria and no bacterial growth), Growth of *Staphylococcus epidermidis* colony on MHA media after incubation 37°C for 24 hours (Positive (+): colonies are showing bacterial growth, Negative (-): No colonies, no inhibition of bacterial growth, and no bacterial growth), Inhibitory against Bacteria (Positive (+): Concentration of the test material has inhibitory properties, Negative (-): Concentration of test material has no inhibition), Positive control using erythromycin antibiotics; Negative control using aquadest

Statistical Test

In the normality test, the results of the data are normally distributed, and the homogeneity test is then performed with the results of non-homogeneous data. Therefore the next analysis test uses the Kruskal Wallis test. Based on the results of the Kruskal Wallis Test, the Asymp Sig (2-tailed) P-value was 0.008 α (0.05) so that significant conclusions can be drawn in the inhibition zone. Green Tea Leaf Extract (*Camellia sinensis*) *epidermidis*

DISCUSSION

Research on green tea leaf extract (*Camellia sinensis* L) is used as an alternative treatment in acne to know the antibacterial effectiveness of ethanol extract of green tea leaves (*Camellia sinensis* L) on the growth of *Staphylococcus epidermidis* bacteria. The effectiveness test starts from the selection of test materials, namely green tea leaves, and extraction are carried out to obtain concentrated extracts of green tea leaves ethanol. This focused extract was divided into several concentrations and then tested on isolates of the bacterium *Staphylococcus epidermidis* ATCC 14990.

Simplisia in powder form was extracted using the maceration method. Maceration is the process of obtaining *Simplicia* using solvents; the solvent used in this study is ethanol, with several shaking or stirring at room temperature (MOH, 2006). Green tea leaf ethanol extract is made at various levels, namely 1.5%, 2.5%, 3.5%, 4.5%, and 5.5%, to determine the ability of each - the growing concentration of test bacteria by diffusion and dilution methods.

The diffusion method is used to determine the sensitivity of bacteria or fungi with the principle of inhibiting the growth of microorganisms marked by the formation of a clear inhibitory zone around the paper disk (disk)⁽⁸⁾. While the dilution method is used to determine the MIC and MBC of the antibacterial, the dilution method's principle is to use a test tube filled with liquid media and several bacteria to be tested, then each filled with diluted material. While the dilution method is used to determine the MIC and MBC of the antibacterial, the principle of the dilution method is to use a test tube filled with liquid media and several bacteria to be tested, then each filled with diluted material.

In this study, work control is also used, namely erythromycin, which functions as a work process control in the study. Work control in the form of erythromycin antibiotics is used to confirm several things, including knowing that the bacterial test isolates are feasible to use, the accuracy of the concentration of bacterial suspensions, the inhibitory zones formed, and the determine the condition of the bacterial growth media used. In addition, based on the ability of erythromycin antibiotics which have anti-inflammatory effects and have an indication in the treatment of acne⁽⁹⁾.

The inhibition zone produced by ethanol extract is almost the same as the erythromycin antibiotic. The formation of inhibition zones indicates that green tea leaves have potential as an antibacterial. The ability to inhibit ethanol extract of green tea leaves is categorized into the ability to inhibit weak, moderate, strong, very strong. In contrast, the ability to inhibit standardized antibiotics such as erythromycin is categorized as sensitive, intermediate, and resistant. Hence, the antibacterial potential of ethanol extract of green tea leaves needs to be further tested before applied broadly and broadly⁽¹⁰⁾.

Based on this study, it can be seen that the extract green tea leaves at a concentration of 5.5% able to kill the *Staphylococcus epidermidis* bacteria. This is known in the absence of the growth of bacterial colonies on media that have been inoculated with test suspension. Whereas at a concentration of 1.5%, 2.5%, 3.5%, 4.5% green tea leaf extract was unable to inhibit the *Staphylococcus epidermidis* bacteria because there was a colony of growth on the media that had been inoculated in the test suspension

In this study, turbidity was not seen clearly because of the color of green tea leaf extract, making it challenging to observe. The suspension of the dilution test solution that had been incubated for 24 hours was inoculated on MHA media to determine the MIC and MBC values. From these results, the lowest concentration of green tea leaf extract, which can kill *Staphylococcus epidermidis*, is at a level of 5.5%, indicating that concentrations below 5.5% do not show inhibition. So, the Minimum Inhibitory Concentration (MIC) of green

tea leaf extract is at a level of 4.5%, and the Minimum Bactericidal Concentration (MBC) of green tea extract is at a concentration of 5.5%

Based on the measurement of inhibition zones by diffusion method in this study, it can be seen that the ethanol extract of green tea leaves at a concentration of 4.5% has the highest inhibitory power against *Staphylococcus epidermidis* bacteria with inhibition zone values of 30 mm. The category of inhibition zone diameter of a natural material against test bacteria can be classified as follows, ≤ 5 mm including the weak type, 6-10 mm moderate, 11-20 mm included in the sharp category, and ≥ 21 mm belongs to the powerful inhibitory category⁽¹¹⁾. Based on the inhibition zone diameter classification, the ability to inhibit ethanol extract of green tea leaves on the growth of *Staphylococcus epidermidis* bacteria is included in the dominant category because the diameter is more than 21 mm.

Catechin compounds are polyphenol compounds present in green tea that are antimicrobial (bacteria and viruses), antioxidants, antiradiation, strengthen blood vessels, smooth urine secretion, and inhibit the growth of cancer cells⁽¹²⁾. The mechanism of action of catechins is by damaging the bacterial membrane. Catechins attach to bacterial membrane lipids and cause aggregation of lipid vesicles so that their fluidity is reduced. This reduced fluidity then causes leakage in the cytoplasmic membrane⁽¹³⁾. Because of this leakage, the materials contained in bacterial cells come out into the extra cells to achieve a balance of effects higher intracellular concentration than extracellular. This imbalance then causes bacterial death⁽¹⁴⁾. Based on the description above prove that green tea leaves have active antibacterial compounds that can inhibit bacterial growth.

CONCLUSION

Based on research that has been done regarding the effectiveness of ethanol extract of green tea leaves on the growth of *Staphylococcus epidermidis* bacteria, it can be concluded as follows:

1. Minimum Inhibitory Concentration (MIC) of green tea leaf extract (*Camellia sinensis* L) against *Staphylococcus epidermidis* is 4.5%, Minimum Bactericidal Concentration (MBC) of ethanol extract of green tea leaves (*Camellia sinensis* L) against *Staphylococcus epidermidis* is 5.5%.
2. Ethanol extract of green tea leaves (*Camellia sinensis* L) effectively inhibits the growth of *Staphylococcus epidermidis* bacteria

REFERENCES

1. Alamsyah AN. Conquer Disease with Green Tea (Taklukan Penyakit dengan Teh Hijau). Jakarta: Agro Media Pustaka; 2006.
2. Winona M, Sulistiyarningsih. Antibiotic resistance in acne treatment therapy (Resistensi antibiotik pada terapi pengobatan jerawat). Farmaka. 2018;16(2).
3. Dilly NP, M Teguh W. Antibacterial effect of Green Tea (*Camellia sinensis*) to *Staphylococcus aureus* in vitro (Uji Efek Antibakteri Infusum Teh Hijau (*Camellia sinensis* L) Terhadap Bakteri *Staphylococcus aureus* In vitro). Jurnal Medikal Planta. 2011;1(3).
4. Kemenkes RI. Basic Health Research 2013 (Riset Kesehatan Dasar Tahun 2013). Jakarta; Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan RI; 2013.
5. Depkes RI. General standard parameters of medicinal plant extracts (Parameter standart umum ekstrak tumbuhan obat). Jakarta: Depkes RI; 2006.
6. Kristanti, et al. Phytochemical Textbook (Buku Ajar Fitokimia). Surabaya: Airlangga University Press; 2008.
7. Sirait M. Phytochemical guides in pharmacy (Penuntun fitokimia dalam farmasi). Bandung: Penerbit ITB; 2007.
8. Harborne JB. Phytochemical Methods: A guide to the modern way of analyzing plants (Metode Fitokimia: Penuntun cara modern menganalisis tumbuhan). translated by Kosasih Padmawinata and Iwang Soediro. Bandung: Penerbit ITB; 2007.
9. Herwin, et al. Antibacterial activities of leaf ethanol extract and green tea (*Camellia sinensis* L.) On bacteria causing acne (*Propionibacterium acne* and *Staphylococcus epidermidis*) by the diffusion method Aktivitas (Antibakteri Ekstrak Etanol Daun Dan Ampas Teh Hijau (*Camellia sinensis* L) Terhadap Bakteri Penyebab Jerawat (*Propionibacterium acne*) Dan *Staphylococcus epidermidis* Secara Difusi Agar). Jurnal Farmasi As-syifaa. Makassar: Fakultas Farmasi Universitas Muslim Indonesia; 2018.
10. Jawetz E, Melnick JL, Adelberg EA. Medical microbiology (Mikrobiologi Kedokteran), translated by Mudihardi E. Jakarta: Penerbit Salemba Medika; 2013.
11. Yulis A, Siva Fauziah. Potential Antibiotics and Diffusion Test in vitro on erythromycin cream formulation (Potensi Antibiotik dan Uji Difusi secara in vitro pada formulasi krim eritromisin). Jurnal Medical Profession. 2019;1(3):277-282.

12. Saraswati FN. Antibacterial Activity Test of 96% Ethanol Extract of Kepok Kuning Banana (*Musa balbisiana*) Skin Waste Against Acne-Causing Bacteria (*Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Propionibacterium acne*) (Uji Aktivitas Antibakteri Ekstrak Etanol 96% Limbah Kulit Pisang Kepok Kuning (*Musa balbisiana*) Terhadap Bakteri Penyebab Jerawat (*Staphylococcus epidermidis*, *Staphylococcus aureus*, dan *Propionibacterium acne*)). Undergraduate Thesis. Jakarta: UIN Syarif Hidayatullah; 2015.
13. Susanto D, Sudrajat, Ruga R. Study of the active ingredients of red meranti (*Shorea leprosula* Miq) as a source of antibacterial compounds (Studi kandungan bahan aktif tumbuhan meranti merah (*Shorea leprosula* Miq) sebagai sumber senyawa antibakteri). Mulawarman Scientific. 2012;11(2):181-190.
14. Radji. Microbiology Textbook, Pharmacy and Medicine Student Guide (Buku Ajar Mikrobiologi Panduan Mahasiswa Farmasi dan Kedokteran). Jakarta: Buku Kedokteran EGC; 2011.