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Microwave-Assisted Isolation of Catechins from Menoreh Green Tea (Camellia sinensis) and Their Antibacterial Efficacy

Isolasi Katekin dari Teh Hijau (*Camellia sinensis*) Menoreh Berbasis Gelombang Mikro serta Evaluasi Efektivitas Antibakterinya

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ABSTRACT

Green tea (Camellia sinensis) is a rich source of bioactive compounds such as catechins, tannins, caffeine, and flavonoids, with catechins known for their antibacterial activity; however, their extraction yield remains relatively low, necessitating optimization. This study investigated the effects of temperature (40 – 60 °C) and extraction time (3 – 9 minutes) using Microwave-Assisted Extraction (MAE) on catechin yield, as well as the antibacterial activity against Staphylococcus aureus, Streptococcus mutans, and Pseudomonas aeruginosa. A total of 5 g of dried green tea powder was extracted with 50 g of 60% ethanol at a microwave power of 450 W, followed by solvent evaporation and catechin analysis using High Performance Liquid Chromatography (HPLC). The results showed that the yield increased with higher temperature and longer extraction time, reaching a maximum of 4.98% at 60 °C for 9 minutes. Meanwhile, antibacterial activity testing using the disc diffusion method showed the largest inhibition zone against P. aeruginosa (19.67 mm), followed by S. aureus (17.50 mm) and S. mutans (12.50 mm). The MIC and MBC values were 3.125% and 6.25%, respectively, with an MBC/MIC ratio ≤ 4, indicating bactericidal activity. These findings confirm that MAE optimization effectively enhances catechin yield while producing extracts with promising potential as natural antibacterial agents.

Keywords: Antibacterial, catechin, green tea, microwave-assisted extraction, Staphylococcus aureus.

ABSTRAK

Daun teh hijau (*Camellia sinensis*) merupakan sumber senyawa bioaktif seperti katekin, tanin, kafein, dan flavonoid, dengan katekin memiliki aktivitas antibakteri, namun rendemen ekstraksinya masih rendah sehingga perlu optimasi. Penelitian ini mengkaji pengaruh suhu (40 – 60 °C) dan waktu (3 – 9 menit) pada *Microwave-Assisted Extraction* (MAE) terhadap rendemen katekin serta aktivitas antibakteri terhadap *Staphylococcus aureus*, *Streptococcus mutans*, dan *Pseudomonas aeruginosa*. Sebanyak 5 g serbuk daun teh diekstraksi dengan 50 g etanol 60% pada daya 450 W, diikuti evaporasi dan analisis dengan *High Performance Liquid Chromatography* (HPLC). Hasil menunjukkan rendemen meningkat dengan suhu dan waktu, dengan nilai tertinggi 4,98% pada 60 °C selama 9 menit. Sementara itu, uji aktivitas antibakteri dengan metode difusi cakram menunjukkan zona hambat terbesar terhadap *P. aeruginosa* (19,67 mm), diikuti *S. aureus* (17,50 mm) dan *S. mutans* (12,50 mm). Nilai MIC dan MBC masing-masing sebesar 3,125% dan 6,25%, dengan rasio MBC/MIC ≤ 4 yang menunjukkan sifat bakterisidal. Hasil ini menunjukkan bahwa optimasi MAE efektif meningkatkan rendemen katekin sekaligus menghasilkan ekstrak dengan aktivitas antibakteri yang potensial sebagai agen alami.

Kata kunci: Antibakteri, katekin, teh hijau, microwave-assisted extraction, Staphylococcus aureus.

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INTRODUCTION

Indonesia is an agricultural country rich in natural resources, including tea plants. Tea, originally from China, has been consumed for thousands of years and remains one of the most widely consumed beverages worldwide. As one of the world's major tea producers, Indonesia had approximately 101,281 hectares of tea plantations in 2022, producing 124,662 tons of tea leaves. However, production slightly declined to 122,700 tons in 2023 (Indonesia Investments Report, 2026).

Green tea leaves (*Camellia sinensis*) are rich in bioactive compounds, including catechins, tannins, caffeine, and flavonoids, which are known to provide various health benefits, particularly antibacterial activity. Among these compounds, catechins have attracted considerable attention due to their potential use in combination therapy with antibiotics for treating resistant *Staphylococcus aureus* infections (Arodes and Hasudungan, 2020). *S. aureus*, a Gram-positive bacterium, has been reported as one of the most susceptible bacteria to plant extracts, showing the largest inhibition zone in the study conducted by Nuryana *et al*. (2024). Nevertheless, under weakened immune conditions, *S. aureus* can become pathogenic and cause various infections, including acne, boils, purulent wounds, and pneumonia (Astutiningsih *et al*., 2014). Consequently, infections caused by *S. aureus* continue to be a major health concern in developing countries, including Indonesia.

Indonesia has several green tea-producing regions, one of which is the Special Region of Yogyakarta. Among its well-known tea-producing areas is the Nglinggo tea plantation in Kulon Progo Regency, which produces Menoreh tea and also serves as a popular agro-tourism destination. In this study, fresh tea shoots from the Menoreh tea plantation were used as the main raw material.

The major bioactive compounds in green tea leaves consist primarily of catechins (30 – 42%) and flavonoids (5 – 10%) (Arya *et al*., 2019). Tea shoot tips contain the highest catechin levels because their cells are still actively dividing, leading to increased production of secondary metabolites, including catechins, which contribute to the antibacterial activity against *S. aureus* (Setiawan *et al*., 2019). The main catechin derivatives found in green tea include epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate (Ahmad *et al*., 2022).

Catechins can be extracted from green tea leaves through various extraction methods. Some commonly used extraction techniques include maceration (Astutiningsih *et al*., 2014; Fajriani and

Djide, 2015; Rustanti, 2018; Mutmainnah *et al*., 2018; Chen *et al*., 2018; Setiawan *et al*., 2019; Arodes and Hasudungan, 2020; Ahmad *et al*., 2022; Alghamdi, 2023; Nuryana *et al*., 2024), brewing (Nur, 2020; Sasmito *et al*., 2020; Putra *et al*., 2020; Liu *et al*., 2022; Allameh and Orsat, 2023), and microwave-assisted extraction (MAE) (Kamaluddin *et al*., 2014; Sari *et al*., 2018; Al-Hatim *et al*., 2022; Ahmad *et al*., 2022; Fujioka *et al*., 2022; Tartillah, 2024). Other methods used for extraction are ultrasonic-assisted extraction (UAE) (Rahman *et al*., 2020; Khasanah *et al*., 2022; Ahmad *et al*., 2023; Rafique *et al*., 2023; Warinhomhoun *et al*., 2024) and subcritical water extraction (SWE) (Hwang *et al*., 2021). Variations in extraction parameters, such as time and temperature, affect the effectiveness of catechin extraction. When the temperature is in the range of 66.7 to 84.7 °C, degradation of the target compound occurs, causing a decrease in catechin content (Kamaluddin *et al*., 2014).

Various advanced processing technologies have been developed as alternatives to conventional extraction methods to improve the efficiency and effectiveness of extracting bioactive compounds from tea leaves (Raghunath *et al*., 2023). Among these technologies, MAE has gained considerable attention due to its shorter extraction time and higher energy efficiency compared with conventional methods such as maceration and brewing. These advantages are mainly attributed to the volumetric heating mechanism of microwaves, which accelerates the diffusion and release of bioactive compounds from the plant matrix. In comparison with other advanced techniques, such as UAE and SWE, MAE also provides a favorable balance between extraction efficiency, operational simplicity, and relatively moderate operating conditions. Although UAE can enhance compound release through cavitation effects, it generally requires longer extraction time, while SWE operates under high temperature and pressure conditions that may lead to the degradation of heat-sensitive compounds.

This research focuses on optimizing controlled operating parameters in MAE to maximize catechin yield while preserving its antibacterial bioactivity. The selection of MAE was also based on the thermal sensitivity of catechins, which are prone to degradation at excessively high temperatures. Therefore, the extraction temperature range was carefully selected to ensure efficient extraction while minimizing the degradation of bioactive compounds. Overall, MAE was considered the most suitable extraction method because it provides an effective balance between extraction efficiency, processing time, and catechin stability, thereby

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enhancing both catechin yield and antibacterial activity.

Several previous studies have investigated extraction parameters such as temperature and extraction time; however, some of the operating conditions applied may promote catechin degradation due to excessive thermal exposure. Moreover, systematic and comprehensive studies evaluating the specific effects of temperature and extraction time on catechin yield remain limited. Therefore, further investigation is necessary to determine the optimal extraction conditions that maximize catechin recovery while minimizing compound degradation.

Furthermore, the relationship between extraction parameters and the antibacterial bioactivity of green tea leaf extract remains insufficiently understood and requires further investigation. Although numerous studies have reported the antibacterial activity of catechins against *S. aureus*, the influence of extraction conditions, particularly temperature and extraction time, on the resulting antibacterial potential has not been comprehensively elucidated. This highlights a knowledge gap between the optimization of the extraction process and the bioactive performance of the obtained extracts. Therefore, this study aimed to systematically evaluate the effects of extraction temperature and time on catechin yield using the MAE method, while also investigating their implications for antibacterial bioactivity.

The extracts obtained from green tea leaves containing catechins were subsequently applied in antibacterial activity tests against *S. aureus*, *S. mutans*, and *P. aeruginosa*. Antibacterial testing was conducted using the Kirby-Bauer disc diffusion method and minimum inhibitory concentration (MIC) analysis, following the approach reported by Hattarki *et al*. (2021). The Kirby-Bauer disc diffusion method was selected because it is widely used, cost-effective, and provides sufficient accuracy in evaluating bacterial susceptibility to antimicrobial compounds.

Overall, this study aimed to determine the effects of extraction time and temperature variations on catechin yield during green tea leaf extraction using MAE, as well as to evaluate the antibacterial bioactivity of the resulting extracts against *S. aureus*, *S. mutans*, and *P. aeruginosa*.

MATERIAL AND METHODS

Equipment and Materials

Fresh young green tea leaves were taken from tea farmers located in the tea plantation of Nglinggo Village, Kulon Progo Regency, D.I. Yogyakarta as the main material in this study. The samples were stored in sealed plastic bags in dry

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conditions, not exposed to light, and stored in a freezer. The chemicals used included pure catechin (Sigma Aldrich), ethanol (Supelco), $FeCl_3$ reagent (Merck), and distilled water obtained from the Bratachem Yogyakarta store. Whatman No. 40 filter paper was used to filter the extract, while MN 827 ATD (Macherey Nagel) disc paper with a diameter of 6 mm for the bacterial inhibition test was obtained from General Labora Yogyakarta.

The main equipment used in this study includes a microwave (Electrolux EMM2308X) as a microwave-assisted extraction device to accelerate the release of active compounds, an oven (Memmert UN 30) for the drying process of tea leaves until they reach a stable water content, and a rotary vacuum evaporator (Heidolph Laborota 4000) to separate and evaporate the solvent efficiently at low pressure to obtain a more concentrated and pure extract. Furthermore, analysis of catechin levels was carried out using HPLC for accurate and precise quantification of compounds.

Methods

Five kilograms of green tea leaves used for testing were cut into smaller pieces, then the moisture content was reduced by drying in an oven at around 40°C for 4 – 5 hours. The dried tea leaves were then ground until fine and sieved through a 30-mesh sieve to obtain tea leaf powder, which was used as the research sample. The samples were weighed before and after the drying process to accurately determine the water content of the tea leaves. Prior to testing, the samples were placed in tightly sealed plastic bags and stored in a freezer to maintain the stability of the bioactive components in the tea leaf powder.

The extraction process was carried out using the MAE method without stirring, where this condition was set as a fixed parameter throughout the experiment. Stirring was not varied in this study, as the heating process in MAE occurs volumetrically, allowing for relatively even heat distribution without the aid of mechanical stirring. The operating conditions of the device were at 450 W power with 60% ethanol solvent and a material to solvent ratio of 1:10 (w/v). Prepared samples, as much as 10 g of tea leaf powder was extracted with temperature variations of 40, 50, and 60 °C and extraction times of 3, 6, and 9 minutes according to the experimental design. After the extraction process, the mixture was left for one hour to reach diffusion equilibrium, then filtered using Whatman No. 40 filter paper to separate the filtrate from the solid residue. The obtained filtrate was then concentrated using a rotary vacuum

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evaporator at a temperature of 50 – 60 °C to evaporate the ethanol, resulting in a more concentrated and relatively pure extract. Catechin yield was calculated to evaluate process efficiency, while catechin content was analyzed using HPLC. The obtained catechin extract was then used for antibacterial bioactivity testing against *S. aureus, S. mutans*, and *P. aeruginosa*.

Antibacterial bioactivity testing was conducted using two methods, namely disc diffusion and determination of the MIC. The disc diffusion method is used to evaluate the ability of compounds to inhibit bacterial growth on solid media. The test bacterial suspension was first prepared to reach standard turbidity (McFarland 0.5) and inoculated evenly on the surface of the Mueller-Hinton Agar (MHA) medium. Sterile paper discs with a diameter of ±6 mm that had been impregnated with sample solutions at certain concentrations were then placed on the surface of the medium, followed by incubation at a temperature of 35 – 37 °C for 18 – 24 hours. Antibacterial bioactivity was indicated by the formation of a clear zone around the disc, the diameter of which was measured as an indicator of the strength of bacterial growth inhibition.

Meanwhile, the MIC method is used to determine the lowest concentration of a sample that is still able to significantly inhibit bacterial growth. This test is carried out by preparing a sample solution in a series of graded concentrations in Mueller-Hinton Broth (MHB) liquid medium. A bacterial suspension with a standard density is then inoculated into each microtiter tube or well containing the test solution and incubated at 35 – 37°C for 18 – 24 hours. The lowest concentration in the dilution series that shows the medium remains clear, without any turbidity due to bacterial growth, is determined as the MIC value. Thus, the disc diffusion method provides a qualitative picture through the inhibition zone, while the MIC method produces more accurate quantitative data regarding the antibacterial effectiveness of a compound.

RESULTS AND DISCUSSION

Catechin Extraction

MAE extraction (450 W; 60% ethanol; 1:10 w/v ratio) was carried out at various temperatures of 40 – 60 °C and for 3 – 9 minutes according to the experimental design, with each treatment performed in duplicate to ensure data reliability and reproducibility. Qualitative testing using $FeCl_3$ reagent was conducted to identify the presence of phenolic compounds, including catechins, in the MAE Menoreh tea leaf extract. As shown in Table 1, all combinations of temperatures (40, 50, and 60 °C) and extraction times (3 – 9 minutes) showed positive results, indicated by a color change from yellowish green to green

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black after the addition of $FeCl_3$. This color change indicates the formation of a complex between the Fe^{3+} ion and the aromatic hydroxyl group in the phenolic compound. The consistency of the results across all treatments indicates that catechins were successfully extracted under various MAE operating conditions without experiencing significant degradation. This indicates that microwave heating effectively accelerates the release of active compounds from the leaf matrix, while maintaining the stability of its phenolic structure. This finding aligns with Nur's (2020) report, which states that catechins exhibit a green black color reaction with $FeCl_3$, a characteristic of the polyphenol group. The presence of phenolic compounds in the extract not only confirms the success of the extraction process but also provides a scientific basis for further antibacterial bioactivity testing, given that catechins are known to work through mechanisms of cell membrane destruction and inhibition of bacterial metabolic enzymes.

Although the $FeCl_3$ test indicates the presence of phenolic compounds, this method is qualitative and cannot be used to ensure that catechin degradation does not occur. However, the increase in catechin yield with increasing temperature and extraction time indicates that within the conditions used (40 – 60 °C), the extraction process is still dominated by diffusion mechanisms without any indication of significant degradation. This is supported by research by Kamaluddin *et al*. (2014) that when the temperature is in the range of 66.7 to 84.7 °C, degradation of the target compound occurs, causing a decrease in catechin content. Therefore, the conditions used in this study are still within a relatively safe range for thermal degradation.

Table 1. Qualitative test of catechin content in green tea with $FeCl_3$ reagent.

No.	Time (minutes)	Temperature (°C)	Original Color	Tested Color
1.	3	40	Yellowish green	Green black
2.		50	Yellowish green	Green black
3.		60	Yellowish green	Green black
4.	6	40	Yellowish green	Green black
5.		50	Yellowish green	Green black
6.		60	Yellowish green	Green black
7.	9	40	Yellowish green	Green black
8.		50	Yellowish green	Green black
9.		60	Yellowish green	Green black

Determination of Catechin Concentration

Analysis of catechin levels was carried out using an HPLC instrument at the UII Integrated Laboratory, with a Shimadzu Prominence LC-20AD UFLC system equipped with an SPD-M20A diode array detector. Determination of catechin levels was carried out at a wavelength of 254 nm. Operational conditions included a flow rate

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of 1 mL/min with an analysis time of 30 minutes. The mobile phase used was a mixture of 0.3% acetic acid (75%) and acetonitrile (25%) with an injection volume of 20 µL on a C18 column. Furthermore, catechin levels were determined by multiplying the sample peak area by the standard curve equation obtained from the standard solution.

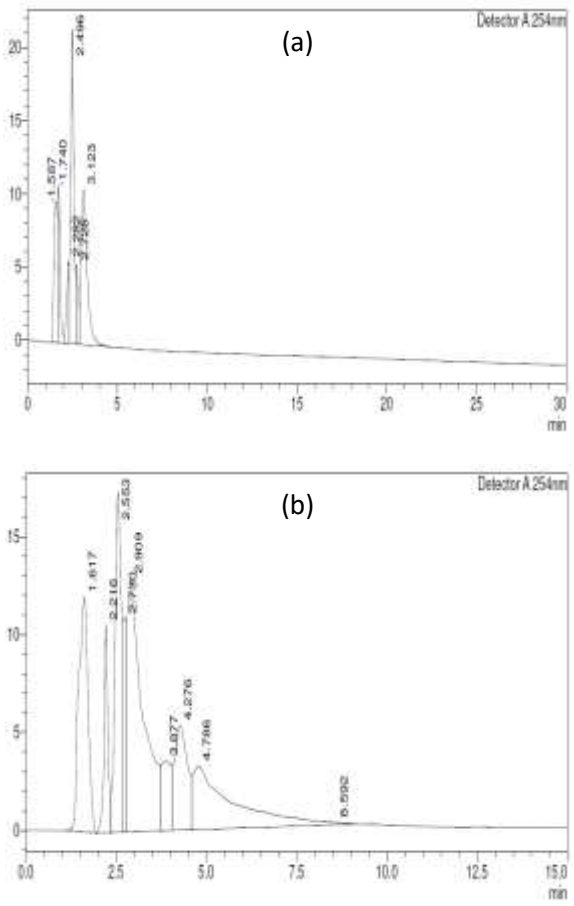

Figure 1. HPLC chromatograms: (a) standard catechin solution, (b) green tea leaf extract sample.

The HPLC chromatogram profile in Figure 1 shows the presence of a catechin peak at a specific retention time consistent between the standard solution and the sample. This retention time agreement confirms the presence of catechin in the tea leaf extract. Furthermore, the sharp and well-separated peak shape indicates that the analytical method has good resolution and minimal interference from other compounds.

Figure 2 shows a calibration curve for measuring catechins based on the relationship between the measured area and the catechin concentration (ppm) in the sample. The regression equation Area = 977.38 × [Catechin] (in ppm) indicates that for every 1 ppm increase in catechin concentration, the measured area will increase by 977.38 units. With an R^2 value of 98.67%, the variation in area can be explained by the catechin concentration, indicating that the relationship between the two variables is very consistent and strong.

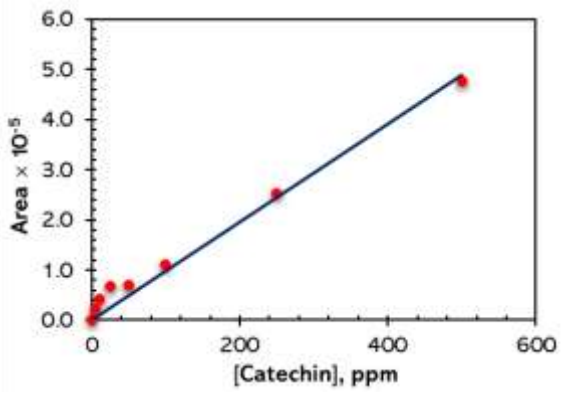

Figure 2. Standard calibration curve of catechin

Catechin Yield

Catechin yield was calculated using equation (1).

$$\text{Yield (\%)} = \frac{W_1}{W_0} \times 100\% \qquad (1)$$

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where W_0 is the initial dry weight of the sample before extraction and W_1 is the weight of the catechin obtained from the extraction, both expressed in mg.

Table 2. Relationship between yield and catechin concentration from HPLC analysis under various extraction conditions.

No	Time (min)	Temp. (°C)	HPLC Area	Conc. (mg/g)	Yield (%)
1	3	40	34182	19.24	1.94
2		50	37969	21.37	2.15
3		60	38715	21.79	2.18
4	6	40	43117	24.27	2.43
5		50	47046	26.48	2.65
6		60	50274	28.30	2.83
7	9	40	53171	29.93	3.01
8		50	54847	30.87	3.11
9		60	87862	49.46	4.98

Table 2 below shows that increasing temperature and extraction time increases the HPLC area value, which is followed by an increase in catechin concentration and yield. At 40 °C for 3 minutes, a catechin concentration of 19.24 mg/g with a yield of 1.94% was obtained, while at 60 °C for 9 minutes, it increased to 49.46 mg/g with a yield of 4.98%. This trend indicates a linear relationship between the HPLC area and catechin concentration, as well as a direct relationship between catechin concentration and the resulting yield. Thus, it can be confirmed that the increase in yield observed in this study reflects not only an increase in the amount of extract, but also an increase in the content of active compounds (catechins) in the extract.

The results of green tea catechin extraction using the MAE method without stirring showed that the catechin yield increased with increasing temperature and extraction time. As seen in Figure 3, at 40 °C the yield increased gradually from 1.94% (3 minutes) to 3.01% (9 minutes). A similar trend was seen at 50 °C, from 2.15% to 3.11%, while at 60 °C the increase was more significant, reaching 4.98% in the 9th minute. This increase indicates that the longer extraction duration allows more time for the catechins to diffuse out of the leaf matrix into the solvent.

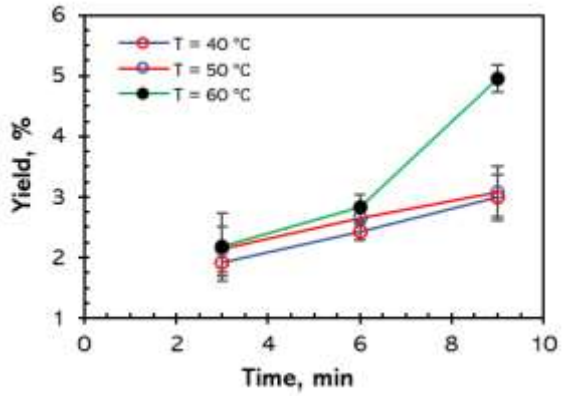

Figure 3. Relationship between catechin yield and extraction time.

Extraction temperature also proved to be a dominant factor in increasing catechin yield. At 3 and 6 minutes, the difference in catechin yield between 40 and 50 °C was relatively small (only about 0.2%), but at 60 °C, a much larger spike was observed, especially at 9 minutes. This is consistent with the theory of diffusion and solubility, where increasing temperature accelerates

the mobility of solvent molecules and increases the diffusion coefficient of bioactive compounds. Therefore, the higher the temperature, the faster the catechins are released from the leaf tissue, although caution should be exercised regarding the risk of phenolic compound degradation if the temperature is too high or the extraction time is too long. In this study, the yield was corrected by subtracting the catechins extracted during the initial heating phase before the target temperature was reached. This correction method is important because in MAE without stirring, some of the catechins begin to dissolve during the heating process, so without correction, the resulting yield could be much higher.

The catechin yield obtained in this study (1.94 – 4.98%) was relatively lower than several previous studies. Liu *et al*. (2022) reported a yield of 21.30% using a conventional heating method with 85% ethanol at 35 °C for 30 minutes. Sukaesih (2022) using a maceration method using ethanol solvent for 30 and 60 minutes obtained yields of 8.22% and 9.44%, respectively. Ahmad *et al*. (2022) used the MAE method for 5 minutes with a yield of 18.53%, while Fujioka *et al*. (2022) reported a yield of 6.64% using MAE at 60 – 80 °C for 5 minutes. This difference may be due to the operating conditions used, namely the extraction process without stirring and the application of corrections to the initial heating phase. This approach produces a more conservative yield value, as it only calculates the extraction contribution at the target temperature conditions, thus avoiding overestimation that can occur during the heating phase. In previous research, no one mentioned whether the yield value obtained had been corrected or not.

Using only pre-corrected data can potentially overestimate the efficiency of the extraction process, as some of the yield may originate from the poorly controlled heating phase. Applying corrected data provides a more accurate representation of extraction kinetics and allows for a more precise evaluation of the effect of temperature on the catechin release mechanism. Practically, this information is highly useful for industrial-scale optimization, as it allows for more precise calculations of energy efficiency and extraction time. Overall, this correction approach ensures that MAE process recommendations are based on valid and reliable scientific data for the sustainable development of catechin extraction technology.

Analysis of Variance (ANOVA)

The results of the descriptive analysis showed that the percent yield varied significantly at each combination of temperature and time tested, with a higher

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average value recorded at 60 °C and 9 minutes (4.98%). Levene's test (test of homogeneity of variance) showed that the variance between groups was homogeneous with a p value = 0.028, which means that the differences between groups can be continued for further analysis without concerns about the problem of inhomogeneity of variance. In addition, temperature has a very significant effect on percent yield, with a partial eta squared of 0.970 based on Table 3. This means that temperature explains about 97% of the variation in percent yield, indicating that temperature is the most influential factor.

Table 3. Result of the ANOVA test of the effect of temperature and time on catechin yield.

Source	Partial Eta Squared
Corrected Model	0.976
Intercept	1.000
Temp_Celcius	0.970
Time_Minute	0.860
Temp_Celcius * Time_Minute	0.666
Error	—
Total	—
Corrected Total	—

a.R Squared = 0.976,
Adjusted R Squared = 0.965

Extraction time also had a significant effect, with a partial eta squared of 0.860. This means that time explained 86% of the variation in percent yield, which also showed a significant effect, although not as large as the effect of temperature. The interaction between temperature and time showed a significant effect, with a partial eta squared of 0.666. This means that the interaction between the two explained 66% of the variation in percent yield, which made a moderate contribution to the variation.

Further tests using Tukey's HSD showed that significant differences were found between 40, 50, and 60 °C at all tested times ($p < 0.001$). Similarly, significant differences were also seen between 3, 6, and 9 minutes ($p < 0.001$). This indicates that both temperature and time independently contribute to the increase in percent yield.

Antibacterial Bioactivity Test

Antibacterial bioactivity tests were conducted on three test microbes, namely *S. aureus*, *S. mutans*, and *P. aeruginosa* using a positive control in the form of chloramphenicol (4 mg/mL) and a negative control in the form of a solvent (water for injection). The positive control showed a high inhibition zone, while the negative control did not show an inhibition zone (0 mm), so it can be confirmed that the observed antibacterial bioactivity comes from the catechin extract.

The test results are shown in Figures 4 and 5 using the disc diffusion method and MIC–MBC determination. In the disc diffusion method, the formation of a clear zone around the disc indicates the inhibition

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of bacterial growth by the active compound. Quantitative data show that *P. aeruginosa* has the largest inhibition zone diameter, namely 19.67 mm, which indicates the strongest antibacterial bioactivity against Gram-negative bacteria. *S. aureus* also showed significant inhibition with a diameter of 17.50 mm, while *S. mutans* had the smallest inhibition zone, namely 12.50 mm.

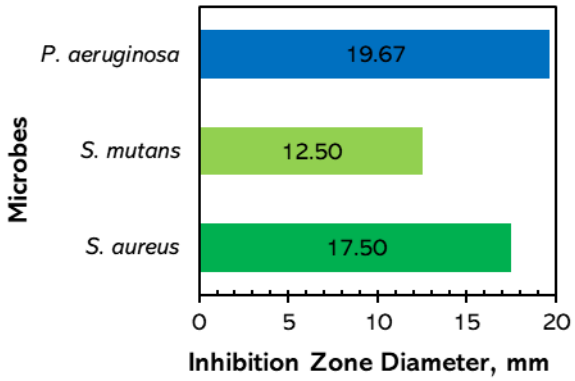

Figure 4. Results of antibacterial bioactivity tests using disc diffusion method.

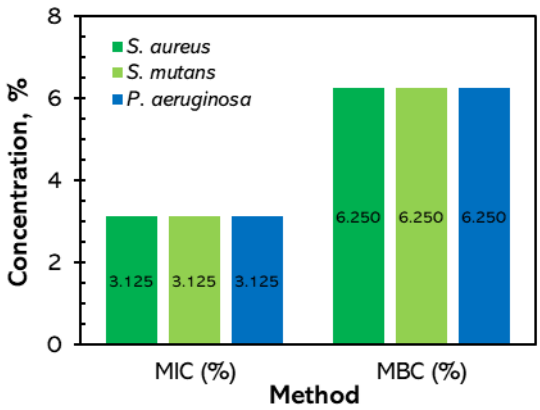

Figure 5. Results of antibacterial bioactivity tests using MIC-MBC method.

These differences in sensitivity levels are closely related to the structural characteristics of each bacterium's cell wall. Variations in peptidoglycan composition, the presence of an outer membrane in Gram-negative bacteria, and cellular permeability factors influence the ability of active compounds to penetrate and disrupt bacterial metabolic systems. Thus, these results confirm that a compound's antibacterial effectiveness is strongly influenced by the biological properties of the target microbe.

Various studies have shown that the extraction method, type of solvent, and operating conditions have a significant effect on the resulting antibacterial bioactivity. The extraction method does not directly affect antibacterial bioactivity but rather plays a role in determining the amount and composition of bioactive compounds in the extract. Variations in extraction conditions, such as temperature, time, and solvent type, affect the efficiency of catechin release from the plant matrix, thus determining the concentration of active compounds that contribute to antibacterial bioactivity. Alghamdi (2023) reported that extraction by maceration method using 80% ethanol at 15°C for 2 days only produced a relatively small inhibition zone against *P. aeruginosa* (1.5 mm) and *S. aureus* (1.6 mm). Different results were shown by Nuryana *et al.* (2024), where the conventional heating method with water solvent at 80 °C for 60 minutes did not provide inhibition against

P. aeruginosa (0 mm), but produced a fairly large inhibition zone against *S. aureus* (9.025 mm). Stronger antibacterial bioactivity occurs in *S. mutans* bacteria. Hattarki *et al*. (2021) obtained an inhibition zone of 28.67 mm, while Annita and Panus (2018) using the maceration method using ethanol solvent at 60 °C reported an inhibition zone of 26.09 mm. For *S. aureus*, Liu *et al*. (2022) through conventional heating with 85% ethanol at 35 °C for 30 minutes produced an inhibition zone of 21.16 mm, while Sukaesih (2022) with ethanol maceration for 60 minutes obtained an inhibition zone of 22 mm.

In general, Gram-positive bacteria are reported to be more sensitive to green tea extract than Gram-negative bacteria such as *P. aeruginosa*. However, as far as the literature used in this study, there are no reports that consistently show that *P. aeruginosa* has the highest inhibitory bioactivity. Differences in antibacterial bioactivity against *P. aeruginosa* can be influenced by the extraction method and operating conditions. Alghamdi (2023) reported that the maceration method with 80% ethanol at 15 °C for 2 days only produced a small inhibition zone against *P. aeruginosa* (1.5 mm). Meanwhile, Nuryana et al. (2024) used a conventional heating method with water solvent at 80 °C for 60 minutes which showed no bioactivity against *P. aeruginosa* (0 mm). In this study, the MAE method with 60% ethanol at 40 – 60 °C for 3 – 9 minutes produced a much larger inhibition zone against *P. aeruginosa* (19.67 mm), indicating that the extraction method and operating conditions significantly influenced the resulting antibacterial bioactivity.

The MIC test results showed that the three test microbes, *S. aureus, S. mutans*, and *P. aeruginosa*, had the same MIC value, namely 3.125%, while the MBC value was 6.25%. The uniformity of the MIC values indicates that the minimum concentration required to inhibit bacterial growth is relatively equivalent in the three species, although differences in sensitivity were still seen in the disc diffusion test through variations in the diameter of the inhibition zone.

The MBC value, which is twice the MIC, indicates that increasing concentration to 6.25% is capable of inducing a bactericidal effect. The MBC/MIC ratio of ≤ 4 further confirms that the test compound is bactericidal, not merely bacteriostatic. This means that at certain concentrations, catechins not only inhibit bacterial growth but also effectively kill bacterial cells.

Overall, the results of this study demonstrate that catechins possess antibacterial bioactivity against *S. aureus, S. mutans*, and *P. aeruginosa*. Relatively low concentrations are sufficient to produce

bacterial inhibition and killing effects. The strongest bioactivity was observed against *P. aeruginosa*, as indicated by the largest inhibition zone diameter. Variations in response between microbes may be caused by differences in cell wall structure, membrane composition, and the intrinsic defense mechanisms of each bacterium. The findings of this study further emphasize the potential of catechins as promising natural antibacterial agents, with strong prospects for further development in pharmaceutical and healthcare applications.

CONCLUSION

Based on the research results, extraction using the MAE method at 450 W power with 60% ethanol as a solvent and varying temperatures and extraction times proved effective in isolating catechins from tea leaves, as demonstrated by the yield values and HPLC analysis results. The obtained extract exhibited significant antibacterial bioactivity against *S. aureus*, *S. mutans*, and *P. aeruginosa*, with the largest inhibition zone diameter for *P. aeruginosa*. The MIC value of 3.125% and MBC of 6.25% indicate that the catechin extract is not only bacteriostatic but also bactericidal at certain concentrations. Overall, the efficiency of MAE coupled with strong antibacterial bioactivity demonstrates the potential of green tea catechins as natural antibacterial agents for further development in the pharmaceutical and healthcare sectors.

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