



# Coconut Shell Charcoal Extract Gel Formulation and Sensitivity Test Against *Escherichia coli* Bacteria

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## Abstract

**Background:** *Escherichia coli* bacteria are normal flora bacteria in the human digestive tract that can turn into opportunistic pathogens and cause diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS). This bacterial infection is generally treated using antibiotics that have been proven to cause resistance in *E. coli*, so it is necessary to treat it using other methods, namely using natural ingredients (herbal medicines) that contain antibacterial compounds such as coconut shell charcoal extract. This research aims to obtain a coconut shell charcoal gel that is most effective in inhibiting the growth of *E. coli* bacteria in vitro. **Method:** The method used in this research was a true experimental post-test-only control group with treatment with different gel concentrations (%) (w/v), namely 3%, 6%, and 9% with repetitions. **Results:** The results of the one-way ANOVA analysis test showed that the treatment given to several concentrations of coconut shell charcoal extract had a mean difference in the area of the inhibition zone for *E. coli* bacteria with a known significance value of 0.002 ( $p < 0.05$ ), so it was continued with the Post Host test LSD and the results obtained from several groups showed significant values ( $p < 0.05$ ) marked with the notation (\*). **Conclusion:** Based on the research results, it can be concluded that the coconut shell charcoal gel with a concentration of 3% (w/v) is most effective in inhibiting the growth of *E. coli* bacteria in vitro.

**Keywords:** Charcoal extract; *E. coli*; Gel formulations; Coconut shell.

## Introduction

*Escherichia coli* bacteria (*E. coli*) is one of the many Gram-negative bacteria with a rod shape, the body is covered with flagella, actively moves without endospores, and round colonies on the surface of Mac Conkey Agar (MCA) media, 2-3 mm in diameter and colored colonies red. Physiologically, these bacteria can reproduce through binary cell division and survive changes in challenging environmental conditions such as temperature, pH, and oxygen levels.

*E. coli* bacteria are bacteria that live naturally (normal flora) in the digestive tract (intestines), which play an essential role in the process of breaking down food (Sutiknowati, 2016). However, under certain conditions, these bacteria can turn into opportunistic pathogenic bacteria when the number of cells is above typical values and are outside the digestive tract, which can cause health problems. *E. coli* bacteria are the leading cause of intestinal infections, characterized by mild to severe (bloody) diarrhea. They are the cause of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS), which humans and animals can experience (Anggraini et al., 2013).

*E. coli* bacterial infection can be transmitted between humans through contact with other people who have been infected or through intermediary animals that carry or contain the bacteria and then transmit it to humans. This bacteria can also be spread through food or drink because *E. coli* can contaminate food or beverages and produce



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toxin compounds that can cause human poisoning (Prasetya et al., 2019).

Washing hands and maintaining cleanliness (sanitation) of food and drinks is the best way to prevent the spread and transmission of *E. coli* bacteria. Hands can be an agent for spreading and transmitting bacteria from one person to another. Sanitation is related to hygiene, that is, people who maintain good hygiene will create a clean and healthy environment to prevent the spread, transmission, and contamination of *E. coli* bacteria (Hutasoit, 2020).

The management and treatment of bacterial infections currently rely on antibiotics. This cannot be separated from the fact that this antibiotic can inhibit (bacteriostatic) and kill (bactericidal) pathogenic bacteria that cause infection. However, the use of antibiotics can cause new problems related to bacterial resistance. The existence of bacterial resistance means that pathogenic bacteria cannot be controlled or controlled easily, and other drugs or higher doses are needed to inhibit or kill them (Roberts et al., 2011).

Reports of *E. coli* bacteria no longer tolerant of antibiotics are very high. The research results of Nurjanah et al. (2020) show that *E. coli* bacteria are resistant to the use of antibiotics from the  $\beta$ -Lactam group. This data is strengthened by the results of research conducted by Normaliska et al. (2019), which showed that *E. coli* bacteria have resistance to antibiotics from the penicillin, cephalosporin, aztreonam, aminoglycoside, trimethoprim-sulfamethoxazole, and quinolones groups.

The use of herbal medicines based on natural ingredients can be used as a solution for treatment, considering that several plants have medicinal properties and antibacterial properties that can help reduce or kill pathogenic bacteria. The use of herbal medicines can be an appropriate alternative because they are considered safer, more accessible, and more affordable than antibiotics made from synthetic ingredients (chemicals) (Prawira et al., 2013).

One of the many nutritious plants currently widely used as herbal medicine is the coconut plant, which has been proven to cure several diseases. Almost all parts of this plant can be used for natural medicine. The fruit's flesh helps treat fever and malaria, while coconut water treats boils, asthma, burns, and constipation (Pritha & Karpagam, 2018).

Coconut shell is a type of agricultural waste with no economic value. Still, it can be used as a raw material for making medicine, where coconut shell extract has sound pharmacological effects (Pritha & Karpagam, 2018). Coconut shell extract contains tannin, saponin, and steroid phytochemical compounds, which can disrupt the life of Gram-positive and harmful bacteria (Mazaya, Karseno, & Yanto, 2020). The research results of Jose et al. (2014) show that coconut shell charcoal extract is effective in inhibiting the growth of *Streptococcus mutans* bacteria, while according to Dwi et al. (2012), coconut shell liquid extract has compounds that play a role in preventing inflammation due to bacterial infections at a concentration of 25 %, 50%, and 100% (v/v).

The presence of active tannin, saponin, and steroid compounds in coconut shell charcoal extract, which have been proven effective in inhibiting the growth of several types of bacteria, has encouraged researchers to research gel formulations to inhibit the growth of *E. coli* bacteria in vitro. This research aims to obtain a coconut shell charcoal extract gel that is most effective in inhibiting the growth of *E. coli* bacteria in vitro.

## Method

### Place and time of research

This research was carried out from September 2021 to February 2022 in 4 different laboratories, namely the Pharmaceutical Biology Laboratory, Pharmaceutical Technology Laboratory, Analytical Chemistry Laboratory, Faculty of Pharmacy and Microbiology and Biochemistry Laboratory, Faculty of Teacher Training and Education, Muhammadiyah University, Purwokerto. This type of research is true experimental research which has five groups, namely a positive control group (chloramphenicol antibiotic) and a negative control

(dimethyl sulfoxide (DMSO) solution), as well as three treatment groups with different extract concentrations, namely P1 (3%), P2 (6 %) P3 (9%) (w/v).

### **Research Sample**

This research used samples of coconut shell charcoal purchased from residents of Nusadadi Village, Nusawungu District, Cilacap Regency, Central Java Province. The sample is then further processed into coconut shell charcoal powder and extracted. The pure culture sample of *E. coli* ATCC 25922 bacteria was obtained from the Integrated Laboratory Building, Microbiology and Biochemistry Laboratory, Faculty of Teacher Training and Education, Muhammadiyah University, Purwokerto.

### **Research procedure**

#### **Coconut Shell Charcoal Extraction**

Extraction of coconut shell charcoal was carried out using the maceration method, which began with the drying process of 10 kg of coconut shell charcoal using a drying cabinet for  $\pm$  2 hours, then sorted, crushed, and sieved, with the final result being 6 kg of coconut shell charcoal powder. After that, 1 kg of charcoal powder was weighed and put into six glass jars, and 1 liter of 96% (v/v) ethanol was added to each jar. The jar was stored at room temperature and closed for five days with observation and stirring daily. If the amount of ethanol in the jar decreases, new ethanol is added until the level mark so that the ethanol volume remains 1 liter. After five days, a filtering process was carried out until liquid and solid filtrate (sediment) of coconut shell charcoal was obtained. After that, the liquid filtrate is extracted using a rotating evaporation device and a water bath to get a thick coconut shell charcoal extract.

Analisis senyawa aktif yang dimiliki oleh ekstrak arang tempurung kelapa dilakukan menggunakan alat *Gas chromatography-mass spectrometry* (GC-MS). Proses ini diawali dengan preparasi sampel dengan cara melarutkan 3 g ekstrak arang tempurung kelapa dengan 3 ml etanol 96% (v/v) pada corong pisah, lalu dihomogenkan selama 5 menit, didiamkan beberapa saat sampai diperoleh dua lapisan berupa fraksi atas dan fraksi bawah dan dipisahkan. Fraksi atas (etanol 96%) (v/v) dikumpulkan pada botol kaca, sedangkan fraksi bawah ditambahkan kembali dengan 3 ml etanol 96% (v/v) dan dilakukan proses dari awal kembali dengan fraksi atas yang telah dikumpulkan dan dipekatkan menggunakan gas nitrogen sampai volumenya hanya tinggal 1 ml.

#### **Preparation of preparations and determination of gel formula**

Several additional ingredients are used to make gel preparations based on coconut shell charcoal extract. This gel formulation was made based on data from previous research literature and carried out trial and error as a pre-formulation step. First, researchers prepared a gel base for coconut shell charcoal extract by dissolving 5% sodium carboxymethyl cellulose (Na-CMC) (w/v) in distilled water on a hot plate while homogenizing (stirring) until dissolved, then adding 10% glycerin (v/v). ), propylene glycol 5% (v/v), and coconut shell charcoal extract 1% (w/v). After that, homogenize until it dissolves and there are no more lumps.

Based on previous reference studies, three recipes were obtained with different amounts of Na-CMC, extract, and distilled water and then tested for homogeneity, spreadability, viscosity, and pH value. Recipes that meet the above requirements are determined as optimal recipes. From this stage, three gel formulas for coconut shell charcoal extract were finally obtained with percentages of 3%, 6%, and 9% (w/v) (Krongrawa et al., 2018; Purnamasari, 2020).

#### **Stability Test of Coconut Shell Charcoal Gel**

Stability test of coconut shell charcoal gel with different concentrations, including homogeneity, spreadability, viscosity, and pH value tests. The homogeneity test was carried

out by applying the gel to a transparent glass surface and observing the formation of coarse grains on the gel.

The viscosity test aims to guarantee the viscosity of the gel. This test is carried out by preparing coconut shell charcoal extract gel to be placed in a viscometer until the spindle is submerged, then setting the speed to 50 rpm.

The spreadability test aims to determine the gel preparation's spread level when applied to the skin. This test was carried out by weighing 0.5 g of the gel, then placing a round glass scale on the top of the gel, giving it a load of 150 g, leaving it for 1 minute, and calculating the spreading power. According to the SNI-06-2588 standard, the optimal spreadability of gel preparations is around 5-7cm (Putri & Anindhita, 2022).

The pH value test is carried out to determine the gel preparation's acid-base level using a pH meter touched to the gel preparation. The pH levels obtained will later be compared with the pH levels of human skin as a control.

### **Test the Effectiveness of Coconut Shell Charcoal Gel Against *E. coli* Bacteria**

The first step that needs to be done is the preparation of *E. coli* bacteria using the Standard Plate Count (SPC) method. Then, 1 ml of *E. coli* bacterial culture was taken to be grown in 25 ml of sterile Tryptic Soy Broth (TSB) media and incubated at 37 °C for 1 x 24 hours. TSB media that had grown bacteria was diluted up to 105 to achieve the right level of accuracy so that the number of cells suspended in the liquid was not too dense, and the number of cells was counted using the turbidimetric method with a wavelength of 600 nm. The absorbance data obtained was then converted into cell number/ml using OD600 Calculator software (<https://www.agilent.com>). Dilution tubes with 108 cells/ml absorbance values are used for antibacterial sensitivity testing.

The antibacterial sensitivity test of coconut shell charcoal gel with test bacteria in the form of *E. coli* bacteria was based on the principle of agar diffusion. Prepare sterile Mueller–Hinton Agar (MHA) media, plates that have been plated with *E. coli* bacterial culture using a spread plate (SP) and five sterile discs of paper, each of which is sequentially smeared with coconut shell charcoal gel with a concentration of 3%, 6% and 9% (w/v), DMSO solution (negative control), and chloramphenicol antibiotic (positive control). The five paper discs were installed on the surface of the MHA media, and the distance between the paper discs was set at the same distance. After that, the MHA media was incubated at 37 °C for 1 x 24 hours, and the formation of an inhibition zone around the paper disc was observed. If an inhibition zone is formed, the diameter is measured using a vernier caliper, and the area of each inhibition zone formed is calculated based on the formula from Kurniawan & Yulistiani (2020).

$$L_{zhaw}: \pi \cdot ra^2$$

$$L_{kic}: \pi \cdot rb^2$$

$$L_{zhak}: L_{zhaw} - L_{kic}$$

#### **Information**

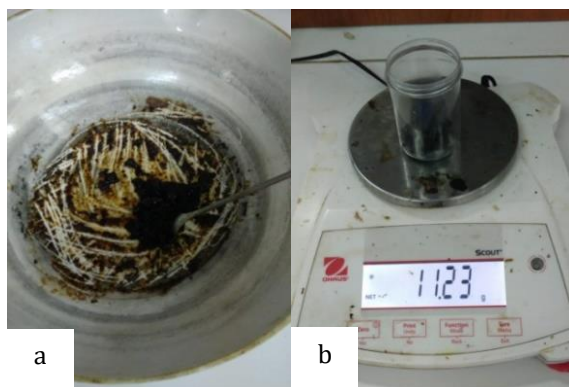
- ra : Radius of inhibition zone (mm)
- rb : Radius of the paper disc (mm)
- Lzhaw : Initial inhibition zone area (mm<sup>2</sup>)
- Lkic : Area of paper disc (mm<sup>2</sup>)
- Lzhak : Area of final inhibition zone (mm<sup>2</sup>)
- π : 3,14

### **Data analysis**

The significance value of the inhibitory power of coconut shell charcoal gel on the growth of *E. coli* bacteria was carried out by statistical calculations using the One-way ANOVA test and the Post Hoc LSD test.

## Result

The extraction results from 6 kg of coconut shell charcoal using 96% ethanol produced a thick extract weighing 11.23 g, as shown in Figure 1. The extraction results were then analyzed for active compound content using the GC-MS method to obtain 731 types of active compounds, with three active compounds being the most dominant, as seen in Table 1.



**Figure 1.** Results of coconut shell charcoal extraction, a) Coconut shell charcoal extract; b) Weight of coconut shell charcoal extract

**Table 1.** Results of GCMS analysis of coconut shell charcoal extract

| TIC Summit report |      |          |       |          |       |      |                      |
|-------------------|------|----------|-------|----------|-------|------|----------------------|
| Summit            | Zone | Zone (%) | High  | High (%) | Z/T   | Mark | Name                 |
| 1                 | TIC  | 367352   | 25,53 | 96067    | 21,35 | 3,82 | Alpha-Santalol (CAS) |
| 2                 | TIC  | 286892   | 19,93 | 76466    | 17,01 | 3,75 | Eicosane (CAS)       |
| 3                 | TIC  | 229534   | 15,94 | 52197    | 11,61 | 4,4  | Santalol (CAS)       |

After learning about the active compounds in coconut shell charcoal extract, The next step is to prepare the gel and determine the optimum formula three times to obtain the data shown in Table 2.

**Table 2.** Coconut shell charcoal extract gel formula tested

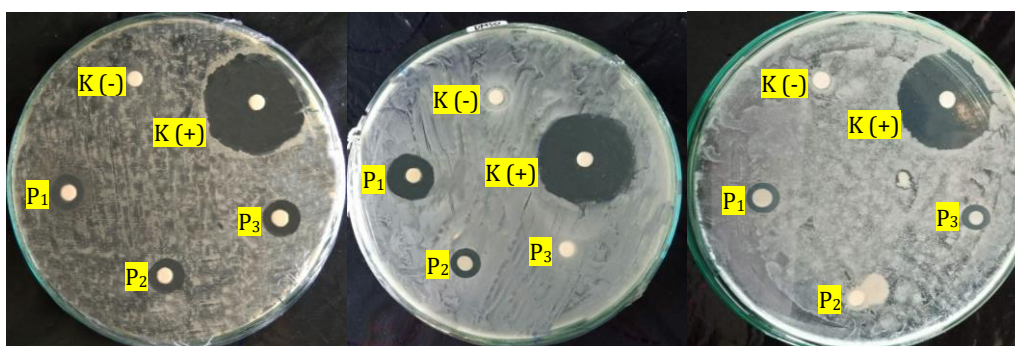
| Composition      | Formula Concentration (%) (w/v) |                     |                     |
|------------------|---------------------------------|---------------------|---------------------|
|                  | P <sub>1</sub> (3%)             | P <sub>2</sub> (6%) | P <sub>3</sub> (9%) |
| Na-CMC           | 0,5 g                           | 0,5 g               | 0,5 g               |
| Glycerin         | 0,5 g                           | 0,5 g               | 0,5 g               |
| Propylene glycol | 1,25 g                          | 1,25 g              | 1,25 g              |
| Extract          | 0,75 g                          | 1,5 g               | 2,25 g              |
| Aquades          | 20 ml                           | 19,25 ml            | 18,5 ml             |
| <b>Amount</b>    | <b>25 ml</b>                    | <b>25 ml</b>        | <b>25 ml</b>        |

Based on the optimum formula that was obtained, the stability of the coconut shell charcoal gel was tested by paying attention to spreadability, homogeneity, viscosity, and pH value with the data presented in Table 3.

**Table 3.** Data from the stability test results of coconut shell charcoal extract gel

| Test Type     | Formula Concentration (%) (b/v) |                     |                     |
|---------------|---------------------------------|---------------------|---------------------|
|               | P <sub>1</sub> (3%)             | P <sub>2</sub> (6%) | P <sub>3</sub> (9%) |
| Spreadability |                                 |                     |                     |
| • TB          | 3,6 cm                          | 3,35 cm             | 3,8 cm              |
| • DB          | 4,35 cm                         | 3,95 cm             | 4,1 cm              |
| Homogeneity   | Homogeneous                     | Homogeneous         | Homogeneous         |
| Viscosity     | 2,424 cP                        | 5,064 cP            | 5,904 cP            |
| pH value      | 6,53                            | 6,98                | 6,94                |

**Information:** TB: no load, DB: with load, cP: centipoise



**Figure 2.** Results of antibacterial sensitivity test against *E. coli* with three repetitions (U1; U2; U3). P1: concentration 3% (w/v), P2: concentration 6% (w/v), P3: concentration 9% (w/v)

**Table 4.** Results of calculating the area of the inhibitory zone of charcoal extract gel from coconut shells against *E. coli* bacteria

| Concentration (%) (b/v) | Inhibition Zone Area (mm <sup>2</sup> ) |                |                | Mean (mm <sup>2</sup> ) |
|-------------------------|---|----------------|----------------|-------------------------|
|                         | U <sub>1</sub>                          | U <sub>2</sub> | U <sub>3</sub> |                         |
| P1 (3%)                 | 83,34                                   | 164,88         | 36,74          | 94,98                   |
| P2 (6%)                 | 38,18                                   | 36,74          | 0              | 24,97                   |
| P3 (9%)                 | 36,54                                   | 0              | 35,32          | 23,95                   |
| K (+)                   | 226,08                                  | 125,6          | 172,7          | 174,79                  |
| K (-)                   | 0                                       | 0              | 0              | 0                       |

**Information:** U1: 1st repetition, U2: 2nd repetition, U3: 3rd repetition. P1: concentration 3% (w/v), P2: concentration 6% (w/v), P3: concentration 9% (w/v)

**Table 5.** Data from calculations of the average area of the inhibition zone between treatments

| Group          | Mean ± SD (mm) | 95% CI          | P            |
|----------------|----------------|-----------------|--------------|
| K (+)          | 174,79 ± 50,27 | 49,90 – 299,67  |              |
| K (-)          | 0,00 ± 0,00    | 0,00 – 0,00     |              |
| P <sub>1</sub> | 94,98 ± 64,85  | -66,13 – 256,10 | <b>0,002</b> |
| P <sub>2</sub> | 24,90 ± 21,58  | -28,71 – 78,52  |              |
| P <sub>3</sub> | 23,95 ± 20,75  | -27,60 – 75,50  |              |

**Information:** P1: 3% extract treatment, P2: 6% extract treatment, and P3: 9% extract treatment.

**Table 6.** LSD Post Hoc Test Results

| Group                            | Mean Difference | Std. Error | Sig    | P      |
|----------------------------------|-----------------|------------|--------|--------|
| K (+) vs K-                      | 174,79          | 31,89      | 0,000* |        |
| K (+) vs P <sub>1</sub>          | 79,80           | 31,89      | 0,031* |        |
| K (+) vs P <sub>2</sub>          | 149,88          | 31,89      | 0,001* |        |
| K (+) vs P <sub>3</sub>          | 150,84          | 31,89      | 0,001* |        |
| K (-) vs P <sub>1</sub>          | -94,98          | 31,89      | 0,014* | < 0,05 |
| K (-) vs P <sub>2</sub>          | -24,90          | 31,89      | 0,453  |        |
| K (-) vs P <sub>3</sub>          | -23,95          | 31,89      | 0,470  |        |
| P <sub>1</sub> vs P <sub>2</sub> | 70,08           | 31,89      | 0,053  |        |
| P <sub>1</sub> vs P <sub>3</sub> | 71,03           | 31,89      | 0,050  |        |
| P <sub>2</sub> vs P <sub>3</sub> | 0,95            | 31,89      | 0,977  |        |

**Information:** The notation (\*) in the table shows significant fundamental differences (P < 0.05)

## Discussion

Coconut shell charcoal extract obtained using the maceration method in this study had a yield value of around 0.0018%. Coconut shell charcoal (Figure 1). The yield value can influence the high or low weight of the extraction results. The weight of the extract will always be directly proportional to the yield value, meaning that if the yield value is high, the weight of the extract obtained will also be more excellent and vice versa (Rizkia, Syaputri, & Tugon, 2022).

Coconut shell charcoal is thought to have active compounds that contain antibacterial compounds. The research results of Mazaya (2020) found that there are phytochemical compounds such as tannins, saponins, and steroids in coconut shell extract that can inhibit bacterial growth.

Coconut shell charcoal extract in this study had three dominant active compounds, namely Alpha-Santalol (CAS), Eicosane (CAS), and Santalol (CAS) (Table 1). The Alpha-Santalol compound, based on the research results of Bommareddy et al. (2019), has been proven to be effective as an antibacterial compound that can suppress the growth of bacteria in both the Gram-positive and Gram-negative groups. This fact is supported by data from research conducted by Kumar et al. (2015) the Santalol compound has antibacterial properties, which effectively control the growth of *E. coli*, *B. subtilis*, *P. aeruginosa*, *S. aureus*, and *S. typhi* bacteria.

Eicosane compounds are also reported to have antibacterial activity. This is based on the research results of Hidayati et al. (2022), which show that the Eicosane compound from *Ceratonia siliqua* extract has antibacterial activity and cytotoxic effects. By knowing the complete content of active compounds in coconut shell charcoal, this material can be proposed as a raw material for herbal medicine against *E. coli* infections.

Based on the presence of antibacterial compounds in coconut shell extract, a gel preparation was formulated to facilitate the antibacterial testing process. The gel formulation in this research underwent a trial-error process first to obtain the optimum formula for coconut shell charcoal gel by modifying the concentration of the gel mass's ingredients. Based on the data in Table 2, the proportion of components used in making the gel is regulated in detail to obtain a suitable gel preparation that matches the characteristics of the specific gel preparation and follows the criteria.

Based on a literature review regarding gel formulations using Na-CMC, concentration modifications were made, such as using 0.5 g of Na-CMC as a gelling agent, which is easily soluble in water so that it can increase the viscosity of the gel preparation (Purnamasari, 2020). 0.5 g glycerin is like a lubricant/moisturizer, effectively used as a humectant and emollient. Use 0.5 g of propylene glycol as a preservative because this material is very stable in an environment with a pH value ranging from 3-6 (Sayuti, 2015).

Table 2 shows that the gel preparation is relatively stable regarding gel viscosity and has a homogeneous shape. These results align with research data from Purnamasari (2020),

which shows that 3%, 6%, and 9% (w/v) gel concentrations are relatively stable in terms of physical and homogeneity.

The results of the gel stability test presented in [Table 3](#) show that the gel spreadability of each concentration is different. The highest spreadability was possessed by gel with a concentration of 3% (w/v) (testing using a 150-gram weight) and 9% (w/v) (testing without using a weight). The concentration of the gelling agent itself dramatically influences the difference in spreadability of a gel preparation. The spreadability of a gel preparation will increase as the concentration of the ingredients increases ([Purnamasari, 2020](#)).

The homogeneity test was carried out to determine the size of the particles contained in the gel preparation. This is based on the fact that the quality of the gel preparation is greatly influenced by the uniformity of the particle size contained in the gel ([Numberi, 2020](#)). This research shows that the gel preparation of coconut shell charcoal extract with three different concentrations has a good level of homogeneity, and no coarse particles were found in the gel preparation.

A good gel preparation must meet the specified viscosity value. The viscosity values of the three gel preparations produced have different values, and the value will be more excellent as the concentration of the gel preparation increases. The gel's viscosity value increase will be higher and directly proportional to the greater concentration of Na-CMC, propylene glycol, and glycerin added to the gel preparation ([Ida & Noer, 2012](#); [Sayuti, 2015](#)). Referring to the SNI standard for gel preparations, which ranges from 2,000-50,000 centipoise (cP), the viscosity values of the three gel preparations have met the SNI standard ([Adhayanti et al., 2022](#)).

Checking the pH value of the gel preparation is carried out to determine the acidic or basic nature, considering that the pH value can affect the gel's ability to control bacteria. The results of measuring the pH values of the three gel preparations show a pH value range of 6.5–6.9, meaning that these preparations have met the gel quality requirements because they are still within the pH value range required by SNI with a pH value of 4.5–8.0 and matches the pH value of human skin which ranges from 4.5–7.5 ([Faradiba et al., 2013](#)).

[Figure 2](#) and [Table 4](#) show that coconut shell charcoal gel can suppress *E. coli* bacteria, as evidenced by forming an inhibitory zone around the paper disc. Of the three gel concentrations tested, the area of the inhibition zone formed varied from each replication. Of the three repetitions, only the three repetitions of the gel with a concentration of 3% (w/v) could control the growth of *E. coli* bacteria. In comparison, the gels with concentrations of 6% and 9% (w/v) had only two repetitions that formed an inhibition zone, while each repetition has no inhibition zone. In general, the results of this research are in line with what was achieved by [Tobing et al. \(2021\)](#), namely that coconut shell charcoal extract was proven to be able to control *E. coli* bacteria in the laboratory, as evidenced by the existence of an inhibition zone around the paper discs. The differences in the area of the inhibitory zone between the three gel concentrations may be caused by several factors, both internal and external. According to [Kurniawan and Aryana \(2017\)](#), several factors can determine the area of the antibacterial inhibition zone, namely incubation time and temperature, method of attaching the paper discs, size of the paper discs, setting the distance between the paper discs, concentration of antibacterial compounds in the extract, composition, and thickness of the media.

The difference in the ability of each concentration of coconut shell charcoal gel to inhibit the growth of *E. coli* bacteria (formation of an inhibition zone) in each replication is probably due to differences in the gel's ability to diffuse into the Muller Hinton Agar (MHA) medium. Gel with a concentration of 3% (w/v) can diffuse quickly into the growth medium because it has a lower viscosity level (more dilute) when compared to gels with a concentration of 6% and 9% (w/v). After diffusing, the 3% (w/v) gel will quickly spread around the disc paper and prevent the growth of *E. coli* bacteria so that the media around the disc paper remains free from bacterial growth and a clear zone is visible. [Muzafri \(2019\)](#) stated that determining whether an inhibition zone exists is the ability or diffusion speed of the antibacterial material being tested.



There are two reasons why gel with a concentration of 3% (w/v) can inhibit the growth of *E. coli* bacteria in all three replications, and it also has the largest inhibition zone area. The first reason is the pH value of the 3% (w/v) gel, which is at the minimum threshold of the environmental pH value of *E. coli* bacteria, namely pH 6.5. pH is one of the environmental factors that can determine the growth ability of bacteria. Generally, bacteria live in an environment with a neutral pH value (7), so when the pH value decreases to acid, bacteria cannot grow. According to Philip et al. (2018), *E. coli* bacteria can grow at pH values between 6.5 and 7.5. The second reason is the low viscosity value of the 3% gel with a value of 2.424 cP, which makes it easier for the active compound to diffuse and come into direct contact with the test bacteria. This aligns with research (Duma et al., 2020; Suhesti, 2021), which states that gel preparations' viscosity value can influence active compounds' effectiveness in inhibiting bacterial growth. The lower the viscosity value, the wider the inhibition zone formed.

Gels with concentrations of 6% and 9% (w/v) could only inhibit the growth of *E. coli* bacteria with a small (narrow) inhibition zone area. Apart from being challenging to diffuse and spread into the growth medium, the antibacterial compounds contained in the 6% and 9% (w/v) gels have difficulty spreading and diffusing so that no or only a few antibacterial compounds can come into direct contact with *E. coli* bacteria and this will result in a narrow zone of inhibition. This is in line with the findings of (Rosyada et al., 2023), which stated that the ingredients making up the gel prevent antibacterial compounds from coming into direct contact with bacteria so that the resulting inhibitory response is small.

Compared with the area of the inhibition zone of chloramphenicol (K+), the area of the inhibition zone of coconut shell charcoal gel is smaller. This is thought to be related to the concentration of the three extracts used being too small (<10% (w/v)), so the area of the inhibition zone is also tiny. This is under the Kemenkes (2020), which states that the active compound content of chloramphenicol capsules ranges from 90.0% (w/v) to 120.0% (w/v), while the concentration of coconut shell charcoal extract used is only 3%, 6% and 9% (w/v) or ten times smaller than the concentration of chloramphenicol capsules used. The main reason the gel concentration is low is to make the gel easier and easier to absorb when applied in vivo.

The sensitivity test results of the DMSO (K-) solution showed no inhibitory activity, as evidenced by the absence of an inhibitory zone around the paper disc. Following the procedures issued by The Clinical & Laboratory Standards Institute (CLSI), the use of DMSO solution as a negative control in antibiotic sensitivity tests is the recommended gold standard, considering that this solution is neutral and does not contain active compounds that can inhibit bacterial growth (Assidqi, Tjahjaningsih, & Sigit, 2012). This follows the results of research by Huda et al. (2019), which shows that DMSO solution cannot inhibit bacterial growth, considering that this solution does not contain antibacterial compounds. DMSO is a solvent capable of dissolving almost all types of polar and non-polar compounds but does not affect the growth of bacteria around the disc paper.

Based on the One-Way ANOVA statistical analysis data, it can be seen that the treatments given to several concentrations of coconut shell charcoal extract had differences in the average area of the inhibition zone for *E. coli* bacteria with a known significance value of 0.002 ( $p < 0.05$ ). After getting significant results, the Post Host LSD test was then carried out to determine whether or not there were significant differences between groups. Based on the results of the Post Host LSD follow-up test, several groups showed substantial values ( $p < 0.05$ ) marked with the appropriate notation (\*) (Table 6). The chloramphenicol (K+) group turned out to be significantly different from the negative control group (K-) and the 3%, 6%, and 9% (w/v) extract treatment groups, while the negative control group was significantly different from the 3% extract experimental group (b/v) only. However, the control groups with experimental extracts of 3%, 6%, and 9% (w/v) were not significantly different.

## Conclusions

Based on the results and discussion in this research, a conclusion can be drawn that the most effective coconut shell charcoal gel in inhibiting the growth of *E. coli* bacteria in vitro is a gel with a concentration of 3% (w/v). Apart from the results obtained, this research was designed from the start so that the results could be applied in vivo. Hence, the gel concentration used was relatively low and very far from the concentration of the positive control. Apart from that, this research directly used gel, without being preceded by research on crude coconut shell charcoal extract on test bacteria, the researchers only based it on literature studies on coconut shell charcoal extract, so the scientific foundation was not strong enough. Due to this problem, preliminary research can be carried out in the future regarding the effectiveness of crude coconut shell charcoal extract in inhibiting the growth of the test bacteria.

## Declaration statement

The authors reported no potential conflict of interest.

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