Potentials of CaO powder result of calcination from green shells (*Perna viridis*), scallops (*Placuna placenta*), and blood clams (*Anadara granosa*) as antibacterial agent

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**Abstract.** Calcium oxide (CaO) can be formed from the calcination process of calcium carbonate or CaCO₃. One of the ingredients that contain quite a lot of CaCO₃ compounds is clam shells. Shellfish shells can be an alternative for making natural antibacterials. Calcination was carried out at 1000°C for 6 hours. The bacteria tested were *Staphylococcus aureus* and *Escherichia coli* which are pathogenic bacteria. The concentration of each CaO powder is 3.5% with a size of 150 mesh. The purpose of this study was to determine the antibacterial effect of CaO from three different shells, namely blood clams, green clams and scallops and to determine the third antibacterial effect of mussels. The research method used was a completely randomized design (CRD) with one factor influencing the antibacterial activity of CaO three times. Parametric data were analysed using ANOVA and Honest Significant Difference (HSD). The research treatment was the use of three different shells, namely blood, scallops and green mussels with tetracycline as control. Parameters observed were pH, calcium, chemical composition, and activity. The pH value of CaO powder has an average of 12.47. The levels of CaO produced were 99.43% in blood clams, 99.62% in green mussels and 98.4% in scallops. The diameter of the inhibition zone of *Staphylococcus aureus* bacteria for each clam shell was 15.22 ± 0.66 mm, green mussel 13.7 ± 0.26 mm and scallop 14.9 ± 0.25 mm. The diameter of the inhibition zone of *Escherichia coli* bacteria produced was 12.2 ± 0.36 mm blood clams, 12.5 ± 0.3 mm green mussels and 13.7 ± 0.2 mm scallops.

1. **Introduction**

Shellfish is one of the most popular seafood, with high nutritional value. Moreover, the quality of cultivated mussels produced by Indonesia is very good, so that there have been many exports of shellfish ranging from whole shells to frozen mussels. KKP through the workshop on Sustainable Aquaculture Development held by the Ministry of National Development Planning/BAPPENAS in 2019, projects that from 2020 to 2024 shellfish production in Indonesia will increase by 50,352 tons. The shells that are caught are mostly taken for the meat, while the shells are discarded or used as crafts. Shellfish shells are widely used as crafts and a mixture of animal feed ingredients [1].

An alternative to the use of clam shells is that it can be used as an antibacterial material. Clam shells contain calcium in the form of calcium carbonate (CaCO₃). CaCO₃ is one of the compounds found in clam shells, generally CaCO₃ is found in limestone. Shellfish shells can be used as an
antibacterial agent through the calcination process. Calcination is also called thermal decomposition or decomposition with temperature [2]. Calcination can be carried out for 4, 6 to 10 hours and at temperatures ranging from 600°C to 1000°C. The best time for the formation of CaO powder is 6-10 hours and the best temperature is 1000°C [3]. CaO powder formed in the calcination process can be used as an antibacterial for both gram-positive and gram-negative bacteria. Calcium oxide (CaO) is one of the metal oxides. Metal oxides are considered to be able to denature microbial cell walls until damage occurs and causes death of microbes. The purpose of this study was to determine the effect of CaO powder as an antibacterial and to determine the best antibacterial from the three types of shellfish.

2. Material and methods
The main ingredients used for the manufacture of CaO powder are green clam shells, scallop shells and blood clam shells obtained from TPI North Jakarta. The size of the clam shells ranged from 6 - 7 cm for green mussels, 5 - 6.7 cm for scallops and 2 - 3.4 cm for blood clams. The condition of the shells is in a clean state where there is very little dirt. The main equipment used is furnace (thermolyne), pH Meter (Denver Instrument), and Xray Flourescence (JEOL Element Analyzer JSX-3211).

2.1. Production of CaO powder
The shells are cleaned and washed to remove any adhering dirt. Then, the clam shells were baked in an oven for 24 hours at 105°C, then the clam shells were blended and filtered with a size of 120 mesh. The clam shell powder was then calcites using a furnace for 6 hours at a temperature of 1000 °C [4].

2.2. Characterization of CaO powder
Testing of the characteristics of CaO powder was carried out to determine the chemical composition of shells using XRF method. The principle of XRF measurement is based on the process of excitation of electrons in the inner atomic shells when the atoms of an element are exposed to X-rays, the electron vacancies will be filled by outer electrons by releasing specific energy for each element. XRF results are in the form of a spectrum of the relationship between excitation energy and X-ray intensity [5]. The procedure carried out is that an X-ray tube is placed in front of the CaO powder sample to be analysed, the sample is irradiated with X-rays, the value of metal constituent elements and the percentage of shell constituent elements will be shown on the screen.

2.3. Acidity of CaO powder
The pH meter is calibrated with a buffer solution. The electrode cells were rinsed with distilled water and immersed in an acidic pH buffer solution, dried and then immersed in an alkaline pH buffer solution. The electrode is dipped into the sample [6].

2.4. Antibacterial activity
Put 1 ose of bacteria into the diluent and homogenized. Then the absorbance was measured using UV-Vis spectrophotometry with a wavelength of 560 nm. 0.2 ml of microbes was taken and put into a petri dish and added 20-25 ml of TSA media. Then homogenized and allowed to solidify. The solid media was perforated using a well then the sample was placed on the media and allowed to stand. Incubated at 30-35°C for 18-24 hours. After incubation, the inhibition zone formed was observed [7] with modification.

The research method used is a completely randomized design (CRD). The study was conducted with 3 repetitions and 3 treatments.
3. Result and discussion

3.1. Chemical composition.

The XRF method is carried out by identifying and enumerating the characteristics of X-rays that occur from the photoelectric effect. The photoelectric effect occurs because the electrons in the target atom (sample) are exposed to high-energy beams (gamma radiation, X-rays).

The content of each element in each shell is certainly different based on the habitat of the shellfish, therefore when the XRF test was carried out it was found that the content of CaO and other elements was different. The results of the XRF test in this study resulted in a higher CaO value in blood clams, namely 99.43% when compared to the results of research conducted by [8], which is 98.63% and produces a higher CaO value in green mussels, which is 99.62% when compared to the other results.

Based on Table 6. There are several elements formed after the calcination process. CaO has a percentage compared to other elements. This is because the main compound that makes up shells is CaO, which when heated at high temperatures will break into CaO and CO2. In addition, several other elements are formed at less than 1% which are included in the metal oxide category. According to [9], the presence of oxide compounds other than CaO may be caused by the presence of other compounds that also react when the synthesis process is carried out.

| Element | Blood Clam | Green Shells | Scallops |
|---------|------------|--------------|----------|
| SO3     | 0.09 %     | 0.10 %       | 0.91 %   |
| Cl      | 0.08 %     | 0.09 %       | 0.06 %   |
| CaO     | 99.43 %    | 99.62 %      | 98.40 %  |
| TiO2    | -          | 0.01 %       | -        |
| MnO     | 0.01 %     | 0.01 %       | -        |
| Fe2O3   | 0.04 %     | 0.01 %       | -        |
| Ag2O    | 0.14 %     | 0.16 %       | 0.12 %   |
| SrO     | 0.22 %     | -            | 0.25 %   |
| Al2O3   | 0.26 %     |              |          |

High temperature and long-time of heating process will result in high CaO content. In this study, a temperature of 1000°C was used with a heating time of 6 hours. The calcination process is generally carried out at a temperature of 900 °C – 1100 °C. The higher the temperature and the longer the calcination process, the higher the CaO content obtained. So as to produce a perfect process without burning CO2 and producing high CaO.

3.2. Acidity test

The CaO powder of this three shells produced an average pH of 12.47 where the CaO powder from the blood clam shells had a pH of 12.47, the CaO powder from the scallop shells was 12.47 and the CaO powder from the green clam shells was 12.45. The pH produced in this study was higher than the pH in the previous study conducted by [9], namely the pH of blood clam shells with an average of 11.3. Some of the factors that influence these differences are due to different types of shellfish species, differences in calcination temperature and differences in particle size of CaO powder.

The alkaline of CaO makes CaO has a high level of alkalinity, it can be used as an inhibitor of bacterial growth. The effect of alkali on antimicrobial activity by CaO hydration is considered to be one of the basic antibacterial mechanisms. Moreover, since the bactercidal activity of CaO powder is greater than NaOH solution at identical pH, the antibacterial mechanism of CaO is not only due to alkalinity but also the action of active oxygen generated from CaO [10].
3.3. Antibacterial activity
Antibacterial testing was carried out to test the antibacterial activity of *Eschericia coli* and *Staphylococcus aureus* against CaO powder from the three shells. This test was carried out by the well method. This method is considered more effective than the disc method. According to [11], the thickness of the filter paper used in the antibacterial test will affect the size of the inhibition zone formed. Therefore, it stated that the use of the well method is better and more effective. Other factors in the inhibition test were bacterial population, antimicrobial concentration, composition of culture media, incubation time and temperature.

Three shells CaO powder used against *Staphylococcus aureus* formed an average inhibition zone of 13.7 mm to 15.2 mm, while against *Escherichia coli* bacteria formed an average inhibition zone of 12.2 mm – 13.7 mm. The resulting inhibition zone includes a strong inhibition zone where it can be said that the use of CaO powder effectively affects the growth of bacteria. Based on [12], the very strong inhibition zone is 20 mm or more, the strong inhibition zone is 11-20 mm, the moderate inhibition zone is 5-10 mm and the weak inhibition zone is 5 mm or less. Factors that can affect the size of the antibacterial inhibition are the type and age of the bacteria, the concentration of antimicrobial substances and the amount of inoculum, the resistance of bacteria to antimicrobial substances related to differences in the cell walls of the test bacteria and the levels of active substances or functional groups of antimicrobial substances. The results of the strong inhibition zone formed prove that CaO is effectively used as an antibacterial.

![Figure 1](image1.png) **Figure 1.** Graphic of *Staphylococcus aureus* Inhibition Zone

![Figure 2](image2.png) **Figure 2.** Graphic of *Escherichia coli* Inhibition Zone

CaO belongs to the category of inorganic antibacterials where this antibacterial generally comes from metal materials, such as Cu, Zn, Au, Ag, Ca, and other metal elements. Several previous studies stated that CaO has a fairly high antibacterial effect compared to MgO and ZnO against Escherichia coli bacteria. The antibacterial mechanism is the interaction with tisol, amino and carboxylic protein groups present in the microbial cell wall. Research conducted by [13], stated that CaO causes the loss of the integrity of the cell structure, which results in changes in cell morphology, so that bacterial cells die.

The inhibition zone formed by the reaction of CaO powder against *Staphylococcus aureus* bacteria is larger than the inhibition zone produced by the reaction by *Escherichia coli*. This is caused by differences in the composition of bacterial cells. Gram-positive bacteria such as *Staphylococcus aureus* have simple cell walls. According to [14], the difference in antibacterial response to gram-positive and gram-negative bacteria is related to the structure of the cell wall, such as the amount of peptidoglycan (presence of receptors, pores, and lipids), cross-linking properties, and autolytic enzyme
activity. This component is a factor that determines the penetration, binding, and activity of antimicrobial compounds. Gram-positive bacteria have cell walls and membranes composed of peptidoglycan, teichoic acid and lipoteichoic acid which can be easily penetrated compared to the complex cell walls of gram-negative bacteria [15]. Escherichia coli bacteria have a thick cell wall in the form of peptidoglycan, which is located between the inner and outer membranes. The outer membrane contained in Escherichia coli bacteria protects bacteria from antibiotics [16].

4. Conclusion
The used of these three shells could inhibit the growth of negative and positive bacteria, but the best antibacterial was produced by blood cockle CaO powder with an inhibition zone value of 15.22 mm. The factors that influence the size of the inhibition zone are habitat, type of shellfish, calcination temperature, and powder particle size. To find out its effectiveness on food ingredients, further tests should be carried out on the product.

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