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Original Research Article

# Antibacterial Activity of Red Ginger (*Zingiber officinale* var. *rubrum*) and Black Turmeric (*Curcuma caesia)* Extracts as Growth Inhibitors of *Klebsiella pneumonia*

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ABSTRACT

Secondary plant metabolites play important role as potent drug candidates against antibacterial-resistant pathogens such as *Klebsiella pneumoniae* which commonly causes pneumonia. Red ginger (*Zingiber officinale* var. *rubrum*) and black turmeric (*Curcuma caesia)* from the Zingiberaceae family are the potential plants as antibacterial agents. This study aimed to determine the ability of antibacterial activity and the inhibition mechanism of red ginger, black ginger and mixed (red ginger and black turmeric) extracts on the growth of *K. pneumoniae*. The method used was *in vitro* testing using the dilution method. Results showed that red ginger, black ginger and mixed ethanol extracts could inhibit *K. pneumoniae* growth at concentrations of 125 µg/mL and 250µg/mL, respectively, with a marked decrease in absorbance values before and after incubation. Further observations on bacterial cell leakage showed that the higher concentration of mixed ethanol extract, red ginger and black turmeric, the higher the leakage of *K. pneumoniae* bacterial cells seen from the increase in absorbance values that could be captured by wavelengths of 260 nm and 280 nm, respectively. Based on scanning electron microscope (SEM), the quantity of *K. pneumoniae* after treating black turmeric, red ginger, and mixed ethanol extracts decreased, and the cell walls became wrinkled and destroyed. Hence, ethanol extract from red ginger and black turmeric can be recommended as an alternative natural antibacterial in inhibiting the growth of *K. pneumoniae,* which causes pneumonia infection.

***Keywords***: Antibacterial resistance, Cell leakage, Dilution, Infectious Disease, Medicinal Plant, SEM

## Introduction

Since ancient times, people have known various plant species that have safe pharmacological effects in the body, and now known as phytotherapy. Phytotherapy is a process of preventing, maintaining health, and also treating a deadly disease using natural plants. Phytotherapy is a process of preventing, maintaining health, and also treating a deadly disease using natural plants.[1]

Nowadays, respiratory infection is one of the biggest causes of death from infectious diseases in children and adults, such as pneumonia. Pneumonia is the biggest infectious cause of death in children compared to other infectious diseases. Pneumonia accounts for 14% of all deaths of children under five years but 22% in children aged 1 to 5 years.[2] Pneumonia is swelling (inflammation) of the tissue in either or both lungs where the alveoli are filled with fluid that will limit oxygen intake. Pneumonia usually caused by a bacterial infection or a virus. It is recorded that 2.1% of the pathogen that causes pneumonia *Klebsiella pneumoniae*.[3]

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The use of antibiotics and vaccines is an attempt to treat diseases caused by bacterial infections. However, in managing respiratory tract infections, the fundamental problem is the involvement of multiple bacteria in the disease, each with specific structural and biochemical characteristics, pathogenicity, and differences in antibiotic resistance.[4]*K. pneumoniae* is a bacteria with high antibiotic resistance rates, making it difficult to choose the right antibiotic for treatment.[5,6] In this situation, the use of phytotherapy becomes a viable solution.

Plants have secondary metabolite compounds that can damage pathogenic bacteria.[6] Secondary metabolites produced by plants have antibacterial abilities and inhibitory mechanisms against bacterial growth. There are mechanisms such as inhibition of bacterial cell wall and protein synthesis, and cell membrane function, which cause bacterial death.[7,8] Such metabolites may act synergistically or with other antibacterial agents (antibiotics).[9]

Of the plants often used in herbal medicine are ginger and turmeric, which have functioned as pain relievers, antibiotics, stimulants, and others functions. Red ginger (*Zingiber officinale* var. *rubrum*) is commonly consumed as a spice and flavouring with many medicinal properties.[10] Red ginger is effective in herbal medicine and may provide suitable leads for future development and clinical utility as an inhibitor of nosocomial pneumonia caused by *Pseudomonas aeruginosa*.[11]

Black turmeric (*Curcuma caesia* Roxb.) comes from the genus *Curcuma*, which has been shown to contain curcumin as an antibacterial. Curcumin can improve anti-*Klebsiella* (hypervirulent *K. pneumoniae)* treatment according to the crucial role of biofilm, capsule and efflux systems in the pathogenesis.[12] Java turmeric (*C. xanthorrhiza* Roxb.) with curcumin also has antibacterial activity against *K. pneumoniae*, which causes pneumonia.[13] Unfortunately, the

antibacterial activity of black turmeric extract against the resistance to multi antibiotics of *K. pneumoniae* has not been investigated. Hence, this study aimed to explore other sources of curcumin as an antibacterial, namely black turmeric combined with red ginger. This study can provide new information about the benefits of red ginger and black turmeric as a source of natural antibacterial against *K. pneumoniae*, which causes pneumonia infection.

## Materials and Methods

*Extraction sample*
Samples of red ginger and black turmeric were obtained from native plants of Riau province plantations, especially in Pekanbaru, Indonesia. Identification was carried out at the Department of Biology, Riau University. Every 2000 grams of black turmeric and fresh red ginger rhizomes were washed, dried, and ground into a fine powder using a blender. The crude ethanol extract was created by combining the filtrates from each extraction, and the leftover solvent was evaporated using a rotary evaporator.[14]

*Phytochemical screening*
Samples of red ginger and black turmeric were used to test for other secondary metabolites such as flavonoids, alkaloids, saponins, phenols, and terpenoids.[15,16]

*Antibacterial activity test*
*Disc Diffusion test.*
The antibacterial activity was evaluated using the disc diffusion test with modifications.[18] Bacterial culture was made by taking as much as 1 needle inoculation of bacterial culture inserted into physiological NaCl solution which is equalized with MC Farland 0.5 standard solution, then bacterial culture was applied on solid media and allowed to dry. The concentrations of viscous extracts of red ginger, black turmeric, and mixed were 20, 40, 60, and 80%. The bacterial suspension was a negative control, and the chloramphenicol antibiotic was a positive control. The growth inhibition zone around the disk was measured to obtain the results, and each experiment was performed three times.

*Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC).*
The antibacterial activity was measured using a spectrophotometer before and after incubation.[19] For 125, 250, 500, and 1000 µL/mL a test tube containing 5 mL of sterile NB medium and 0.5 mL of the extract in each of its four strengths was employed. If there was no turbidity (bacterial OD = 0), the lowest concentration was believed to suppress bacterial growth, and the MIC value was determined accordingly. The MBC value was computed if the measurement results demonstrate that the lowest concentration of the extract has an OD of 0 (no turbidity).

*Leakage of Cellular Metabolites.*
Measurement of cell metabolite release was carried out using an ultraviolet-visible Spectrophotometer (UVS) by measuring absorbance at a wavelength of 260 nm (nucleic acid) and 280 nm (protein). [20] A total of 5 mL of MHB containing 1 mL of bacterial inoculum was added with 1 mL each of extract, positive control (chloramphenicol), and bacterial suspension (as control). The whole test was incubated at 35 ± 20ºC for 24 hours under aerobic conditions. The absorbance of the liquid was directly measured with a spectrophotometer.

*Antibacterial Working Mechanism Testing based on Scanning Electron Microscope (SEM).*
The bacterial culture colony was inoculated into 5 mL of MHB and incubated at 352ºC for 18 hours. Following that, 0.1 mL of culture suspension (1.5x108 cfu/mL) was re-inoculated into 5 mL of MHB and incubated for 12 hours. Borges et al. (2016) treated cell pellets with 5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) and 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4). The samples were dried at ethanol concentrations of 30, 50, 70, 90, and 96%. The dried samples were coated with a layer of gold and tested using a JEOL-JSM-6510LA SEM instrument.

*Statistical analysis*
Data was collected in triplicate and expressed as mean standard deviation. In the Statistical Package for the Social Sciences (SPSS version 16.0), data was assessed using one-way analysis of variance (ANOVA).

## Results and Discussion

Black turmeric (*Curcuma caesia*) and Red ginger (*Zingiber officinale* var. *rubrum*) had an antibacterial activity against *Klebsiella pneumoniae*. *K. pneumoniae* is a species of gram-negative bacteria that can cause pneumonia. *K. pneumoniae* is highly resistant to antibiotics because these bacteria can produce ancarbapenemase enzyme. So the carbapenem class of antibiotics will not work properly to treat the infection and kill the bacteria.[5] This research has found a natural antibiotic as phytotheraphy for infectious *K. pneumoniae* diseases.

*Phytochemical Evaluation and Total Flavonoids Content and Total Phenolics Content*
Phytochemical screening of ethanol extracts from red ginger and black turmeric rhizomes showed the presence of several compounds (Table 1, Figure 1). Both plants' main phytochemical constituents include alkaloids, flavonoids, saponins, tannins, and terpenoids.
The active compounds in both extracts could inhibit *K. pneumonia* growth. The content of flavonoid compounds blocks the synthesis of bacterial DNA.[6] Flavonoids work as antibacterial agents by forming complex compounds against extracellular proteins that impair the integrity of bacterial cell membranes, interfere with microorganism cell activity, and interrupt the microbial cell cycle.[21,22,23] Tannins have properties that suppress the development and action of rumen microorganism proteases by targeting the bacterial cell wall.[24] They can also disrupt the cell layer if they are sufficiently lipophilic.[25] Alkaloids mostly display antimicrobial action through intercalation into bacterial cell walls and DNA.[26] When galangin is combined with amoxicillin, transmission electron microscopy finds detachment of the cell's outer membrane, instruments that may harm the inner peptidoglycan layer.[27] Saponins are also active compounds that have antibacterial activity (Khan et al., 2018). Saponins work by interfering with the surface tension of bacterial cells so that bacterial cells easily leak and lyse.[28]

**Table 1:** Phytochemical content of black turmeric and red ginger ethanol extracts

| Constituent | *Curcuma caesia*Roxb | *Zingiber officinale* var. *rubrum* |
|---|---|---|
| Alkaloid | + | + |
| Flavonoid | + | + |
| Saponin | + | + |
| Tanin | + | + |
| Terpenoid | + | + |

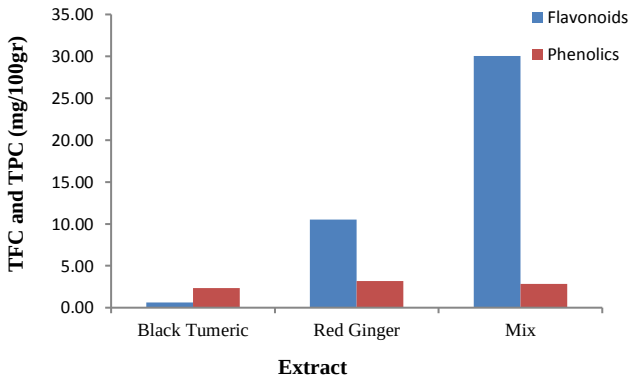

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**Figure 1:** Total flavonoids and total phenolics content of ethanol extract Black turmeric, Red Ginger and Mixed

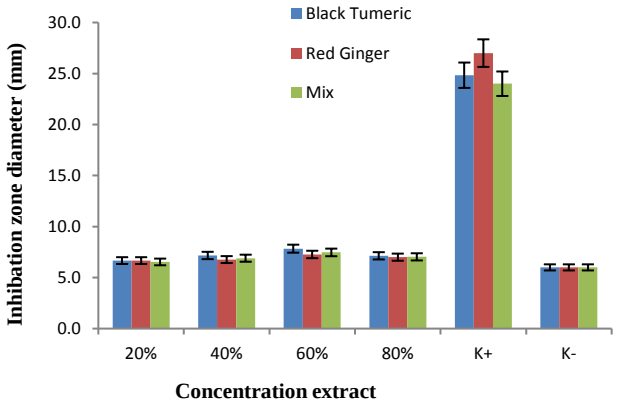


**Figure 2:** Diameter of the zone inhibition of ethanol extracts of black turmeric, red ginger, and mix against the growth of *Klebsiella pneumonia.*

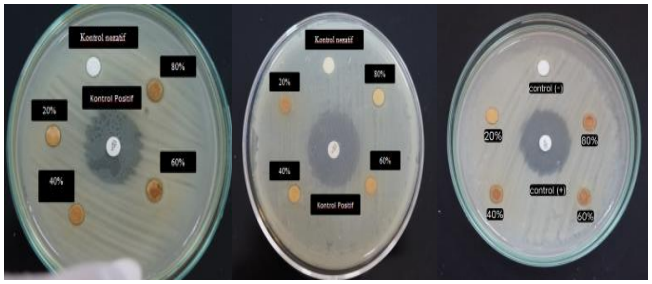

**Figure 3:** Inhibition zone diameter (a) of ethanol extract of black turmeric (b), red ginger, and (c) mix against the growth of *Klebsiella pneumonia*.

*Antibacterial activity*

The antibacterial activity of different extracts of red ginger rhizome and black turmeric rhizome against *Klebsiella pneumoniae* bacterial strains is shown in Table 2. The results reveal the variability of the inhibitory concentration of each extract against certain bacteria. The three samples showed inhibition results against *K. pneumoniae*. However, the inhibition of bacterial growth depended on the concentration level as the inhibitory action of the extracts was found to increase with increasing concentration, as evidenced by a higher zone of inhibition at higher concentrations of each extract (Figure 2).The average diameter of the inhibition zone produced at each extract concentration is 6-9 mm. The inhibition ability is indicated by a clear zone around the disk (Figure 3).

Except for the concentration of 125 g/mL, which nevertheless showed an increase in absorbance value, all concentrations and the control group showed a drop in absorbance value. (Table 2). The decrease in absorbance indicated that the antibacterial activity of the ethanolic extract of black turmeric increased against *K. pneumoniae*. Meanwhile, the concentration of 125 µg/mL still showed the presence of bacterial growth after incubation. So that the concentration of 125 µg/mL does not include MIC because there is no decrease in absorbance or there has been no inhibition of black turmeric extract on the growth of *K. pneumoniae*.

In all concentrations and the positive control group, Table 2 shows a decrease in absorbance value, this decrease in absorbance value indicates that the Minimum Inhibitory Concentration (MIC) value before incubation has decreased, indicating the antibacterial activity of the red ginger ethanol extract increased against *K. pneumoniae.* Table 2 shows a decrease in the absorbance value for each concentration, but at a concentration of 1000 µg/mL, there was an increase in absorbance. This may be because the administration of antibacterial in excessive amounts will cause bacterial cells to become immune and resistant so that bacteria are still growing.[29]

**Table 2:** Minimum Inhibitory Concentration (MIC) test results of black turmeric extract and red ginger ethanol against K. pneumonia

| Extract | Concentration | MIC mean | | Mean Difference | Note | Sig |
|---|---|---|---|---|---|---|
| | | Before | After | | | |
| Black turmeric | 125 | 0.24 ± 0.01 | 0.26 ± 0.01 | 0.021 | increase | 0.04 |
| | 250 | 0.32 ± 0.02 | 0.29 ± 0.03 | -0.03* | decrease | |
| | 500 | 0.28 ± 0.01 | 0.26 ± 0.002 | -0.02* | decrease | |
| | 1000 | 0.31 ± 0.01 | 0.28 ± 0.02 | -0.03* | decrease | |
| | K + | 0.32 ± 0.02 | 0.27 ± 0.04 | 0.07* | decrease | |
| | K - | 0.38 ± 0.03 | 0.51 ± 0.12 | 0.13 | increase | |
| Red ginger | 125 | 0.43 ± 0.02 | 0.36 ± 0.01 | -0.070* | decrease | 0.00 |
| | 250 | 0.39 ± 0.03 | 0.29 ± 0.03 | -0.100* | decrease | |
| | 500 | 0.35 ± 0.02 | 0.32 ± 0.02 | -0.030* | decrease | |
| | 1000 | 0.31 ± 0.03 | 0.29 ± 0.04 | -0.020* | decrease | |
| | K+ | 0.32 ± 0.04 | 0.30 ± 0.04 | -0.020* | decrease | |
| | K - | 0.19 ± 0.30 | 0.31 ± 0.03 | 0.120 | increase | |
| Black turmeric and Red ginger | 125 | 0.38 ± 0.03 | 0.25 ± 0.02 | -0.130* | decrease | |
| | 250 | 0.46 ± 0.09 | 0.31 ± 0.04 | -0.150* | decrease | 0.00 |
| | 500 | 0.54 ± 0.07 | 0.39 ± 0.03 | -0.150* | decrease | |
| | 1000 | 0.39 ± 0.01 | 0.43 ± 0.01 | -0.04 | increase | |
| | K + | 0.38 ± 0.02 | 0.32 ± 0.02 | -0.060* | decreas | |
| | K - | 0.54 ± 0.03 | 0.62 ± 0.03 | 0.080 | increase | |

Note: * There is a significant difference to the negative control

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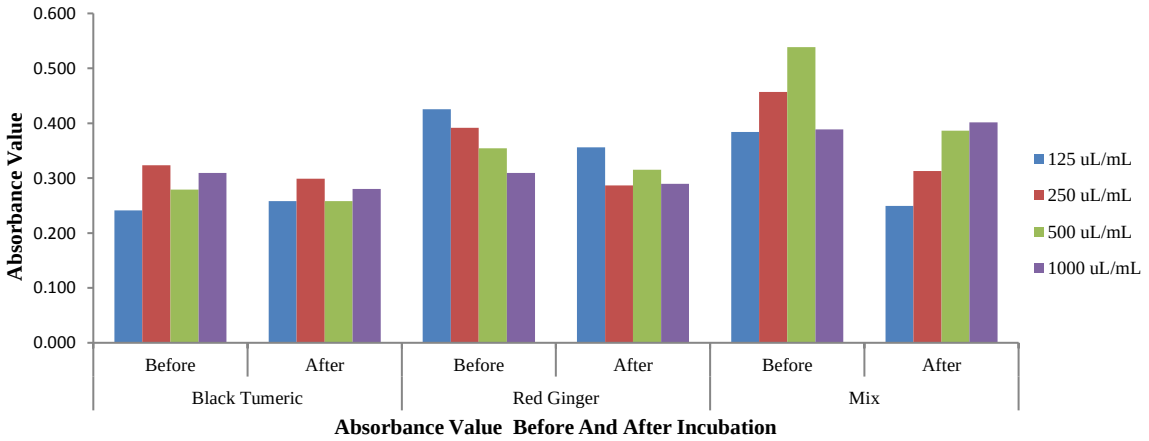


**Figure 4:** Comparison line diagram of the average absorbance value of *K. pneumonia* with black turmeric, red ginger extract and mix before and after incubation.

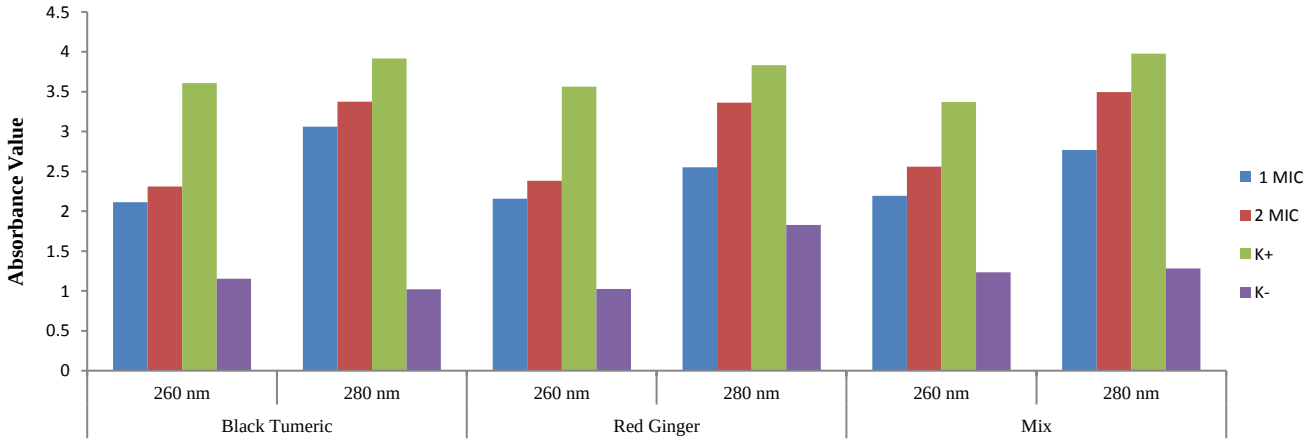


**Figure 5:** Comparative graph of the leakage of *K. pneumoniae* cell contents due to the addition of mixed ethanol extract, black turmeric, red ginger and mix at 260 nm and 280 nm.

**Table 3:** MIC of black turmeric extract, red ginger, mix against *K. pneumoniae*

| Bacteria | Extract (µg/mL) | | |
|---|---|---|---|
| | Black turmeric | Red ginger | Mix |
| *Klebsiella pneumonia* | 250 | 125 | 125 |

Comparison of absorbance values of extracts of black turmeric, red ginger, and mix before and after incubation are shown in Figures (4). Figure 4 shows the average value of the absorbance of *K. pneumoniae* bacteria before incubation in the treatment of black turmeric, red ginger, and mix extracts. In black turmeric extract, there was an increase in the absorbance value the higher the concentration used. While in red ginger extract, there was a decrease in absorbance value, in mixed extracts, there was an increase in absorbance value. Still, at the highest concentration, the absorbance value decreased. The solubility of the extract against the solvent can cause the absorbance value to increase or decrease.

Figure 4 shows that the average absorbance value of black turmeric extract increased at a concentration of 125 µg/mL. Bacterial growth still causes the absorbance value after incubation to increase. Meanwhile, the absorbance value decreased at 250µg/mL, 500 µg/mL, and 1000 µg/mL. This indicates that secondary metabolites contained in black turmeric extract have inhibited *K. pneumoniae* growth.

In the red ginger extract, the absorbance value decreased at the lowest concentration and continued to decrease in the absorbance value until the highest concentration. This indicates that the antibacterial activity of the red ginger ethanol extract increases with the increase in the concentration level used.

In the mixed extract, the absorbance value decreased, but at the concentration of 1000 µg/mL, there was an increase in the absorbance value. This increase in absorbance value may be due to the administration of excessive antibacterial, which will cause bacterial cells to become immune and resistant so that bacteria are still growing.[30]

Minimum Inhibitory Concentrations of ethanol extract of black turmeric, red ginger, and mix on the growth of *K. pneumoniae* bacteria can be seen in Table 3.

The most potent minimum inhibitory concentration was shown by mixed ethanol extract at a concentration of 125 µg/mL with a difference in absorbance value before and after incubation of -0.130, followed by Red Ginger extract at 125 µg/mL with an absorbance value of -0.07. Meanwhile, in the ethanolic extract of black turmeric, the MIC occurred at a concentration of 250 µg/mL with an absorbance value of -0.03. The MIC is indicated by the inhibition of bacterial growth at the smallest concentration, which can be seen from the decrease in the absorbance value before and after incubation.[29] Increasing absorbance value indicates that *K. pneumoniae* is still growing, so the concentration that has increased the absorbance value is not included as the MIC. The Mix ethanol extract sample produced a smaller absorbance value when compared to the red ginger and black turmeric extract samples. This is

due to differences in the active compounds content in the extract. To determine the MIC value, all tubes that did not show turbidity were replanted into NA Broth media. The results of absorbance values after incubation in all tubes are shown in Table 4.

The MIC in the ethanol extract of black turmeric, red ginger and the mixture resulted in an absorbance value > 0, which means that the growth of *K. pneumoniae* was still occurring in the nutrient broth medium. So, it can be said that the ethanolic extract of black turmeric, red ginger and mixture is not bactericidal but only bacteriostatic, which can inhibit the *K. pneumoniae* growth and has not been able to kill *K. pneumoniae*. The results of the leakage of cell contents whose data is captured by the wavelengths of 260 nm and 280 nm are shown in Figures 5.

Figure 5 shows that the extracts of black turmeric, red ginger, mix, and positive control had significant differences from the negative control, where the negative control used ethanol solution, and there was no inhibition so that the bacteria could grow well with a marked increase in the absorbance value. This proves that the inhibition in *K. pneumoniae* is indeed caused by secondary metabolic compounds in the extract. The T-Paired statistical test results also proved a significant difference in concentration treatment on the growth of *K. pneumoniae* bacteria before and after incubation ($P<0.05$). *Zingiberaceae* such as *Curcuma* and *Zingiber* genera contain essential oils (EO) that have been proven to be antibacterial.[31] Essential oils xanthorrhizol from *C. xanthorrhiza* had antibacterial activity against the *K. pneumoniae*.[32] Red ginger contains oleoresins and essential oils with antimicrobial potential. Oleoresin is a resin and essential oil blend. Oleoresin exerts antibacterial activity by denaturing proteins and causing damage to bacteria's cytoplasmic membrane.[10]

Leakage of bacterial cell contents at an absorbance of 280 nm in extracts of black turmeric and red ginger and mix with concentrations of 1 MIC and 2 MICs of 3.061 and 3.376, respectively. The red ginger extract was 2.552 and 3.363, while the mixed extract was 2.769 and 3.493, respectively. In the positive control, the leakage of cell contents was 3.915 (black turmeric control), 3.831 (red ginger control), and 3.978 (mix control). While in the negative control, the leakage of cell contents was 1.023 (black turmeric control), 1.827 (red ginger control), and 1.283 (mix control) (Figure 5B).

ANOVA statistical analysis, this indicates that the addition of mixed ethanol extract, red ginger, and black turmeric had a significant effect on cell leakage in *K. pneumoniae*. LSD analysis showed that adding mixed ethanol extract, red ginger, and black turmeric at 125 µg/mL and 250 µg/mL, and positive control chloramphenicol gave a p-value <0.05 compared to without adding extract. This shows a significant difference in cell leakage at 260 nm and 280 nm waves with mixed ethanol extract, red ginger, and black turmeric compared with mixed ethanol extract, red ginger, and black turmeric. The Pearson correlation test shows that the leakage correlation coefficient at 260 nm is in the range of 0.8-1.0, which means there is a very strong positive correlation where the higher the concentration of mixed ethanol extract, red ginger, and black turmeric has given, the higher the cell leakage at 260 nm and 280 nm wavelengths.

The ANOVA statistical analysis results showed a significant value for the type of extract (< 0.05), so it could be concluded that the three types of extracts influenced the growth of *K. pneumoniae*. The significant concentration result is 0.27 > 0.05, indicating no difference in the mean growth between the three types of extracts. Meanwhile, the significant interaction between extracts and concentrations was 0.01 <0.05, so it can be concluded that there was an interaction between extracts and concentrations in inhibiting *K. pneumoniae* growth.

The antibacterial activity of red ginger, black turmeric and mixed ethanol extract, can cause cell leakage, resulting in bacterial death. Absorbance detection of nucleic acids and proteins outside the bacterial cell indicates that the cell has leaked due to damage to the cell wall or changes in the permeability of the cell membrane so that the bacteria die.[33,34] Absorbance measurements for each sample showed that the absorbance value was directly proportional to the change in the antibacterial concentration used. The higher the test concentration, the higher the absorbance value obtained.[29] In addition, there was a difference in the pattern of increased absorbance in *K. pneumoniae* cells. Namely, the absorbance of 280 nm was higher than 260 nm, indicating that more protein was lost from the cells than nucleic acids. This shows a change in the number of components capable of absorbing light at 260 nm and 280 nm wavelengths due to antibacterial activity. This follows the statement of Naufalin et al.,[35] which explains that the material that can absorb light at wavelengths of 260 nm and 280 nm comes from inside bacterial cells that leak due to antibacterial activity, so the higher the concentration of antibacterial used the amount of material in the number of cells released will also increase.

Antibacterial activity can restrict bacterial development through a variety of ways, including leaking in bacterial cells, which breaks the bacterial membrane as a result of antibacterial activity.[36] This analysis was carried out by observing the increase in absorbance values at a wavelength of 260 nm for nucleic acids and 280 nm for proteins. The wavelength of 260 nm can detect purines, pyrimidines, and ribonucleotides, while the wavelength of 280 nm can detect tyrosine and tryptophan. The compounds that gave absorption at a wavelength of 260 nm were RNA and DNA, while at a wavelength of 280 nm were identified as proteins. The release of nucleic acids and proteins indicates that the cell has leaked due to cell wall breakdown or a change in cell membrane permeability, causing the bacterium to die. Red ginger, black turmeric extract and mix inhibited the growth of *K. pneumonia* bacteria with MIC values at concentrations of 250 (µg/mL), and 125 (µg/mL), respectively. The inhibitory ability can be seen by measuring the cell leakage value at a wavelength of 260 nm (nucleic acid) and 280 nm (protein).

The results of the anti-bacterial mechanism test on bacterial cell wall damage can be seen using a scanning electron microscope. Figure 6 shows that the ethanol extracts of red ginger, black turmeric, and mix inhibited bacteria growth. The damage to bacterial cell wall indicates this. SEM examination results against *K. pneumoniae* bacteria not exposed to ethanol extract showed that bacterial cells of *K. pneumoniae* cells did not experience morphological damage to the cell wall. The growth of *K. pneumoniae* bacteria looks tight with the condition of the cell wall (Figures 6a-6e). SEM examination of *K. pneumoniae* bacteria exposed to ethanol extracts of black turmeric, red ginger, and a mixture of both showed that cells experienced morphological changes in rough cell wall morphology due to cell wall shrinkage and the presence of damaged cell walls, so the cytoplasm came out. The mixed extracts of black turmeric and red ginger provide a good antibacterial effect.

The number of *K. pneumoniae* after treatment with ethanol extracts of black turmeric, red ginger, and a mixture of both decreased, and the cell walls became wrinkled and destroyed. The shape and size of cells were also damaged, and many materials were attached to the bacteria's surface. The surface roughness of the cell wall and the appearance of grooves in the cell wall alter. Incubation with greater doses resulted in cell wall leakage. The bacterial cell wall covers the cytoplasmic membrane, keeps the cell shape, and keeps osmosis pressure from causing lysis. If the cell wall fails to function properly, the cell will lyse as the surrounding hypoosmotic fluid diffuses into the cell, causing swelling.[37, 38]

The extracts of red ginger and black turmeric evaluated in this study showed different levels of antibacterial activity. Therefore, it was concluded that other secondary metabolites present in both plants were responsible for this activity. Another aspect of the antibacterial research in this study was the concentration-dependent inhibition of bacterial growth. The phytochemical results obtained in both extracts have active compounds, namely, flavonoids, alkaloids, saponins, tannins, and terpenoids.[39,40,41] The presence of active compounds in the extract causes leakage of bacterial cells.[42]

Phytotherapy is a crucial link in modern therapy, enabling professionals to conduct additional research on previously known medicinal plants in order to uncover novel remedies. In this case, the solution may be a more efficient commercialization of native flora specific to each country. However, there are still flaws in this study, such as the use of crude extracts, which means that specific chemicals that hinder the development of test bacteria cannot be identified. According to the findings of the study, red ginger and black turmeric extracts could be utilized as a natural antibacterial in preventing the growth of *K. pneumoniae*, the bacteria that causes pneumonia infection.

**Table 4:** MIC of black turmeric extract, red ginger and mix against *K. pneumoniae* bacteria

| Bacteria | Extract (µg/mL) | | | |
|---|---|---|---|---|
| | Concentration | Black turmeric | Red ginger | Mix |
| *Klebsiella pneumonia* | 125 | - | 0.73 | 0.53 |
| | 250 | 0.45 | 0.62 | 0.47 |
| | 500 | 0.37 | 0.51 | 0.44 |
| | 1000 | 0.28 | 0.38 | 0.57 |

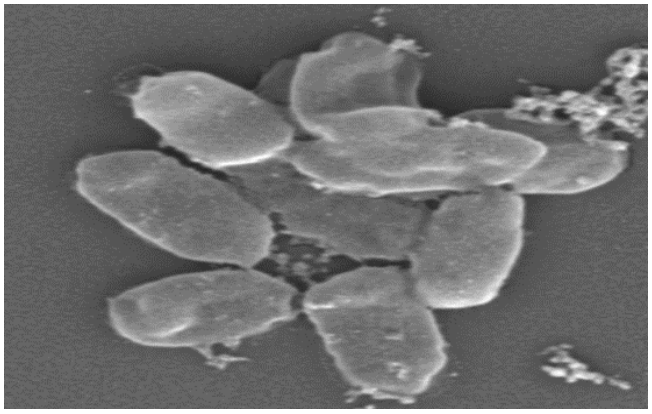

**Figure 6a:** Scanning electron microscope photos. Morphological changes in *K. pneumonia* cells, negative control

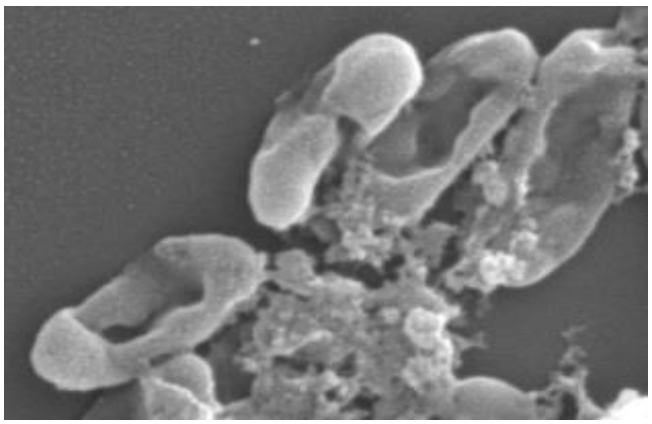

**Figure 6b:** Scanning electron microscope photos. Morphological changes in *K. pneumonia* cells. positive control (Chloramphenicol)

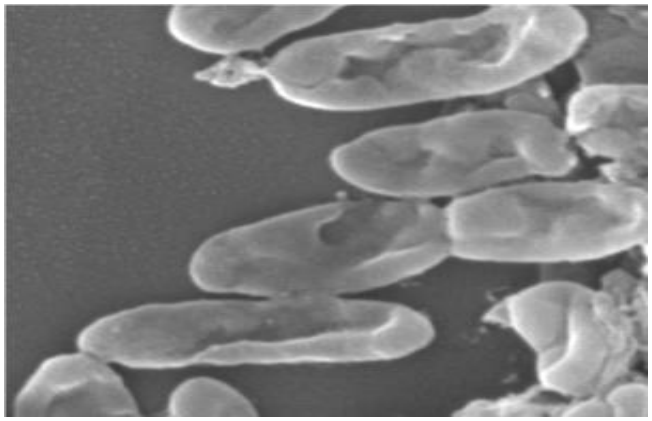

**Figure 6c:** Scanning electron microscope photos. Morphological changes in *K. pneumonia* cells, treatment with black turmeric ethanol extract

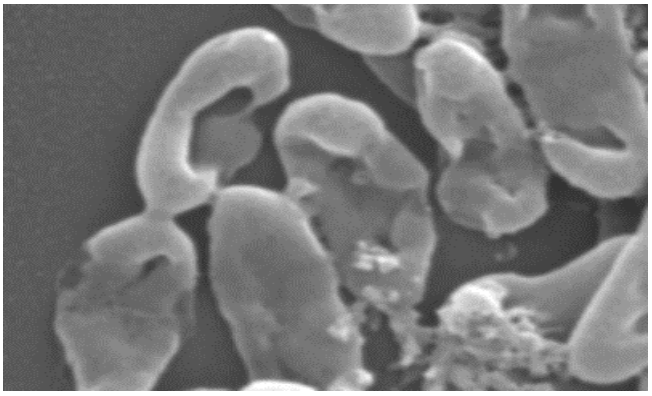

**Figure 6d:** Scanning electron microscope photos. Morphological changes in *K. pneumonia* cells, treatment with mixed ethanol extract

**Figure 6e:** Scanning electron microscope photos. Morphological changes in *K. pneumonia* cells, treatment with red ginger ethanol extract

## Conclusion

Ethanol extracts of red ginger, black ginger and mix could inhibit the growth of *K. pneumoniae* at concentrations of 125 µg/mL and 250µg/mL with a marked decrease in absorbance values before and after incubation. Further observations on bacterial cell leakage showed that the higher the concentration of mixed ethanol extract, red ginger and black turmeric, the higher the leakage of *K. pneumoniae* bacterial cells seen from the increase in absorbance values that could be captured by wavelengths of 260 nm and 280 nm. According to SEM findings, the quantity of *K. pneumoniae* decreased after treatment with ethanol extracts of black turmeric, red ginger, and a combination of both, and cell walls crumpled and were destroyed. According to the findings of the study, red ginger and black turmeric extracts could be utilized as a natural antibacterial in preventing the growth of *K. pneumoniae*, the bacteria that causes pneumonia infection.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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