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.

Key words: 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]

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 flavoring 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 filtrates from each extraction were combined to produce the crude ethanol extract, and the remaining 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 farlan 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 test was carried out using the spectrophotometer method before and after incubation.[19] A test tube containing 5 ml sterile NB medium and 0.5 ml of the extract in each of its four concentrations was used for 125 µL/mL, 250 µL/mL, 500 µL/mL, and 1000 µL/mL, 500 µL/mL. If there was no turbidity (bacterial OD is 0), it was assumed that the lowest concentration inhibits bacterial growth, and the MIC value was calculated accordingly. If the measurement results show that the lowest concentration of the extract has an OD of 0 (no turbidity), the MBC value was calculated.

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 control). The whole test was incubated at 35 ± 20 °C 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 352°C 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 expressed as mean ± standard deviation and carried out in triplicate. Data was tested using one-way analysis of variance (ANOVA) in the Statistical Package for the Social Sciences (SPSS version 16.0).

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]

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] Comparison of absorbance values of extracts of black turmeric, red ginger, and mix before and after incubation are shown in Figures (4). Figure 4A 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 4B 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 5A 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 (Figure 6). 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.

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 they will bear any liability for claims relating to the content of this article.

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References

1. ChassagneF, SamarakoonT, PorrasG, LylesJT, Dettweiler M, MarquezL, Salam AM, Shabih S, FarrokhiDR, QuaveCL. A systematic review of plants with antibacterial activities: A taxonomic and phylogenetic perspective. Front Pharm.2020; 11, 586548.
2. WHO. Penumonia. [Online]. 2021 [cited 2023 May 27]. Available from: https://www.who.int/news-room/fact-sheets/detail/pneumonia.
3. Martin, Rebekah M, Michael A, Bachman. Colonization, infection, and the accessory genome of *Klebsiella Pneumoniae*. Front CellInfect Microbiol,2018; 8(Jan): 1-15.
4. Dutu LE, Popescu ML, Purdel CN, IlieEI, Luta EA, Costea L, GîrdCE. Traditional medicinal plants: a possible source of antibacterial activity on respiratory diseases induced by *Chlamydia pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae* and *Moraxella catarrhalis*.Diversity, 2022; 14: 145-155.
5. TsereteliM, Sidamonidze K, Tsereteli D, Malania L, Vashakidze E. Epidemiology of carbapenem-resistant *Klebsiella pneumoniae* in intensive care units of multiprofile hospitals in Tbilisi, Georgia. Georg,Med News,2018;280(281): 164-168.
6. GorlenkoCL, Kiselev HY, Budanova EV, Zamyatnin AA, Jr Ikryannikova LN. Plant secondary metabolites in the battle of drugs and drug-resistant bacteria: new heroes or worse clones of antibiotics?.Antibiotics (Basel), 2020; 9(4): 170-182.
7. AbdurrahmanM, LestariES ,Prihatiningsih T. The effectiveness of ethanol extract of Neem leaf (*Azadirachta indica*) mouthwash against the growth of *Streptococcus* sp. J BiomediTranslat Res, 2022; 8(2): 76-80.
8. NarayananZ, Glick BR..Secondary metabolites produced by plant bacterial endophytes. Microorganism*s*, 2022;10(10): 2008.
9. WHO. Lack of innovation set to undermine antibiotic performance and health gains [Online]. 2022 [cited 2023 May 27]. Available from: https://www.who.int/news/item/22-06-2022-22-06-2022-lack-of-innovation-set-to-undermine-antibiotic-performance-and-health-gains
10. Assegaf S, Kawilarang AP, Handajani R. Antibacterial activity test of red ginger extract (*Zingiber officinale* var. *rubrum*) against *Streptococcus pyogenes* in vitro. Biomol Health Sci J, 2020;*3*(1): 24-27.
11. Chakotiya AS, NarulaA ,Dharma RK.Efficacy of methanol extract of Zingiber officinale rhizome against acute pneumonia caused by *Pseudomonas aeruginosa*. J Lung Health Disea, 2018; 2(1): 1-8.
12. ZaeandiMK, Ghale HEG, Ranjbar R. Characterization of virulence factors and antibacterial activity of curcumin in hypervirulent *Klebsiella pneumoniae*. Future Med,2022;17(7).
13. SylvesterWS, Son R, Liew KF, RukayadiY. Antibacteri activity of Java turmeric (*Curcuma xanthorrhiza*Roxb.) extract against *Klebsiella pneumoniae* isolated from several vegetables. Int Food Res J, 2015; 22(15):1770-1776.
14. IndrawatiI, Mia M, Isy'ainiRM. Antibacterial activity of ethanolic extracts of rhizome from three ginger varieties against acne isolated bacteria. Nusantara Biosci, 2017; 9(1),.

15. CiulciI. Methodology for the analysis of vegetable drugs, chemical industries branch, division of industrial operations. UNIDO Romania; 1994. 24, 26, 67p.
16. TankoY, Abdelaziz MM, Adelaiye AB, Fatihu MY, Musa KY. Effects of hydromethanolic leaves extract of *Indigofera pulchra* on blood glucose levels of normoglycemic and alloxan-induced diabetic wistar rats. Int J Appl Res Prod Nat, 2008; 1(4):13-18.
17. Pandey D. Antibacterial efficacy of *Curcuma caesia* from bastar district of chhattisgarh, india. *Int J Pharm Sci*,2014; *5*(6): 2294-2301.
18. Abu Bakar MF, Ismail NA, Isha A, Mei Ling AL. Phytochemical composition and biological activities of selected wild berries (Rubus moluccanus L., *R. fraxinifolius*Poir., and *R. alpestris* Blume). Evid Based Complementary Altern Med, 2016; 2016: 1-16.
19. Winnett V, Boyer H, Sirdaarta J, Cock IE. The potential of *Tasmannia lanceolata* as a natural preservative and medicinal agent: antimicrobial activity and toxicity. Pharmacog Com, 2014; 4(1): 42-52.
20. Jenie BSL, Priosoeryanto BP, Syarief R,Rekso GT. Mode of action Temukunci (*Kaempferia pandurata*) essential oil on *E. coli* K1. 1 cell determined by leakage of material cell and salt tolerance assays. HAYATI J Biosci, 2008; 15(2): 56-60.
21. Morgan M, Rogers C, Juan AV, Katherine BG, Anne FP, Morgan TMG, Maureen R, John PC. Cranberry (*Vaccinium macrocarpon*) oligosaccharides decrease biofilm formation by uropathogenic*Escherichia coli*. J Funct Foods, 2017; 17(12): 235-242.
22. Shamsudin NF, Ahmed QU, Mahmood S, Ali Shah SA, Khatib A, Mukhtar S, Alsharif M, Parveen H, Zakaria ZA. Antibacterial effects of flavonoids and their structure-activity relationship study: a comparative interpretation. Molecules, 2022; 27: 1149
23. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. Medicines (Basel), 2018; *5*(3): 93-102.
24. Kaczmarek B. Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials-a minireview. Materials (Basel), 2020; 13(14), 3224.
25. Wojnicz D, Tichaczek-Goska D, Korzekwa K, Kicia M, Hendrich AB. Study of the impact of cranberry extract on the virulence factors and biofilm formation by Enterococcus faecalis strains isolated from urinary tract infections. Int J Food Sci Nut, 2016; 67(8): 1005-16.
26. Mohanan S, Nabeela R, Bimal RKS. Formulation and evaluation of antimicrobial gels for the treatment of paronychia. Int J Appl Pharmaceutics, 2018; 10(6): 161-170.
27. Alsheikh HMA, Sultan I, Kumar V, Rather IA, Al-Sheikh H, Tasleem JA, Haq QMR. Plant-based phytochemicals as possible alternative to antibiotics in combating bacterial drug resistance. Antibiotics (Basel), 2020: 9(8): 480.
28. Sartika D, Astuti S, IswandariR. Inhibitory study of cassava leather ethanol extract as natural antimicrobial in reducing *Salmonella* sp. and *Escherichia Coli* on contamination chicken meat (*Gallus Domesticus*). J Physics: Conf Series, 2021; 1751: 012048.
29. Mere KJ, Bintang M, Safithri M. Antibacterial effectiveness of *Syzygiumcumini* (L.) skeels leaves to*Escherichia coli* pBR322. Indonesian J Chem Res, 2021; 9(1): 8-14.
30. Kowalska KB, Dudek WR. The minimum inhibitory concentration of antibiotics: methods, interpretation, clinical relevance. Pathogens, 2021; 10(2): 165.
31. Dosoky NS, Setzer WN. Chemical composition and biological activities of essential oils of curcuma species. Nutrients, 2018; 10: 1196.
32. Septama AW, Tasfiyati AN, Kristiana R, Jaisi A. Chemical profiles of essential oil from Javanese turmeric (*Curcuma xanthorrhiza*Roxb.), evaluation of its antibacterial and

antibiofilm activities against selected clinical isolates. South Afr J Bot, 2022; 146:728-734.
33. Nobiola RK, Triwahyuni T, Triswanti N, WarganegaraE. Test the sensitivity of yellow turmeric and white turmeric to milk contaminating bacteria. J Ilmu Kesehatan, 2020; 1(4), 263-269.
34. Huang S, Chen X, Yan R, Huang M, Chen D. Isolation, identification and antibacterial mechanism of the main antibacterial component from pickled and dried mustard (*Brassica juncea* Coss. var. *foliosa* Bailey). Molecules, 2022; 27(8): 2418.
35. Naufalin R, Herastuti SR. Antibacterial acitvity of *Nicolaia speciosa* fruit extract. Int Food Res J, 2017; 24(1): 379-385.
36. Benfield AH, Henriques ST. Mode-of-action of antimicrobial peptides: membrane disruption vs. intracellular mechanisms. Front Med Tech, 2020; 2: 610997.
37. Chikada T, Kanai, T., Hayashi, M., Kasai, T., Oshima, T., &Shiomi, D. Direct observation of conversion from walled cells to wall-deficient l-form and vice versa in *Escherichia coli* indicates the essentiality of the outer membrane for proliferation of l-form cells. Front Microbiol, 2021;12, 645965.
38. GórniakI, Rafal B, KróliczewskiJ. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev, 2019; 18(33):241–72.
39. Ita BN, Eduok SI. Antioxidant and Antibacterial Activity of Alkaloid Fractions of TristemmahirtumP. Beauv. Trop J Nat Prod Res. 2020; 4(4):179-184.
40. Khan MI, Ahhmed A, Shin JH, Baek JS, Kim MY, Kim JD. Green tea seed isolated saponins exerts antibacterial effects against various strains of gram positive and gram negative bacteria, a comprehensive study in vitro and in vivo. Evid Based Complementary Altern Med, 2018; 2018, 3486106.
41. Julianti TB, Bakar MFA, Wikantyasning ER. Phytochemical, Antioxidant Analysis and In Vitro Xanthine Oxidase Inhibitory Activity of *Kaempferia parviflora and Kaempferia galanga*. Trop J Nat Prod Res. 2022; 6(12):1981-1985.
42. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards advances in medicinal plant antimicrobial activity: a review study on challenges and future perspectives. Microorganisms, 2021; 9(10): 2041.

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

Constituent	Reagent	*Curcuma caesia*Roxb	*Zingiber officinale* var. *rubrum*
Alkaloid	Mayer	-	-
	Bouchard	+	-
	Wagner	+	+
	Dragondorf	+	+
Flavonoid		+	+
Saponin		+	+
Tanin		+	+
Terpenoid		+	+

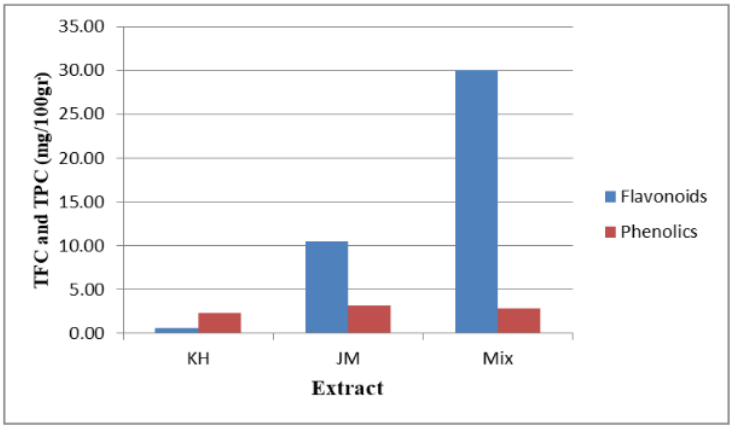


Figure 1. Total flavonoids and total phenolics content of ethanol extract KH (black turmeric), JM (red ginger), and Mixed

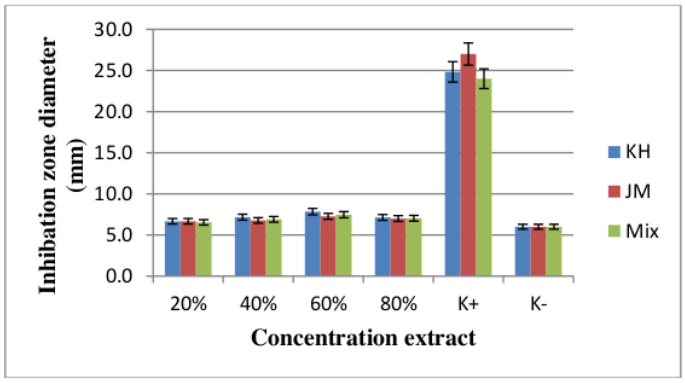


Figure 2. Diameter of the zone inhibition of ethanol extracts of black turmeric, red ginger, and mix against the growth of *Klebsiella pneumonia*.

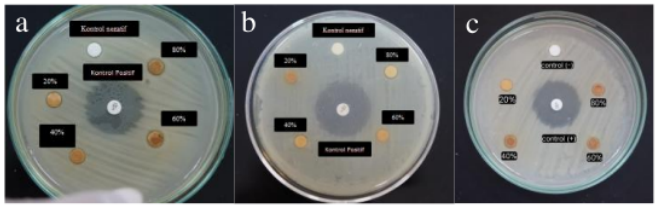


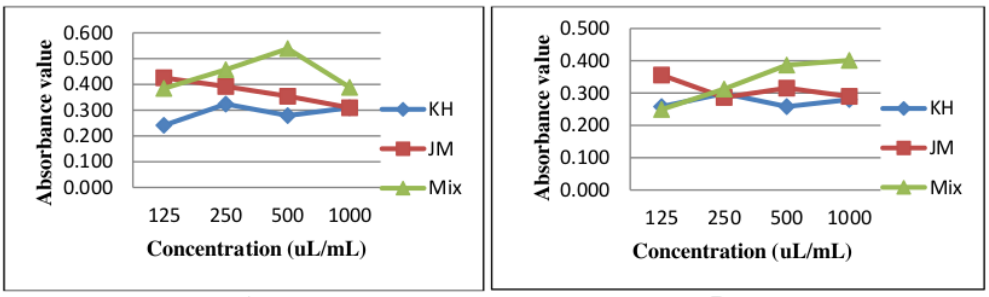
Figure 3.Inhibition zone diameter (a) of ethanol extract of black turmeric (b), red ginger, and (c) mix against the growth of *Klebsiella pneumonia*.

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

Extract	Concentration	MIC mean	Note	Sig

		Before	After	Mean Difference		
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



A B

Figure 4. Comparison line diagram of the average absorbance value of *K. pneumonia* with KH (black turmeric), JM (red ginger) extract and mix. A. before incubation and B. after incubation.

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**

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

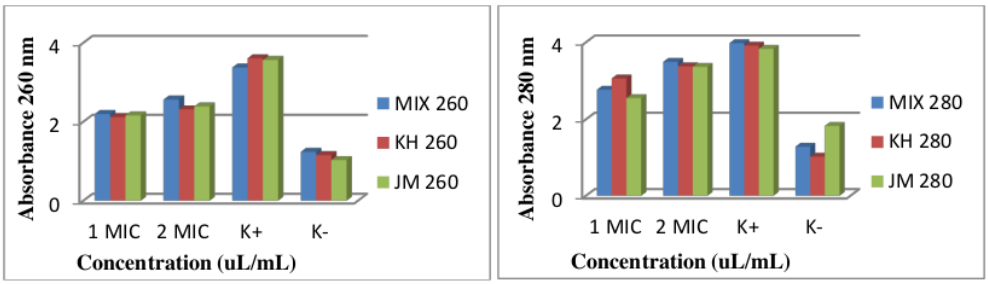


Figure 5. Comparative graph of the leakage of *K. pneumoniae* cell contents due to the addition of mixed ethanol extract, KH (black turmeric) and JM (red ginger) at, A. 260 nm and B. 280 nm.

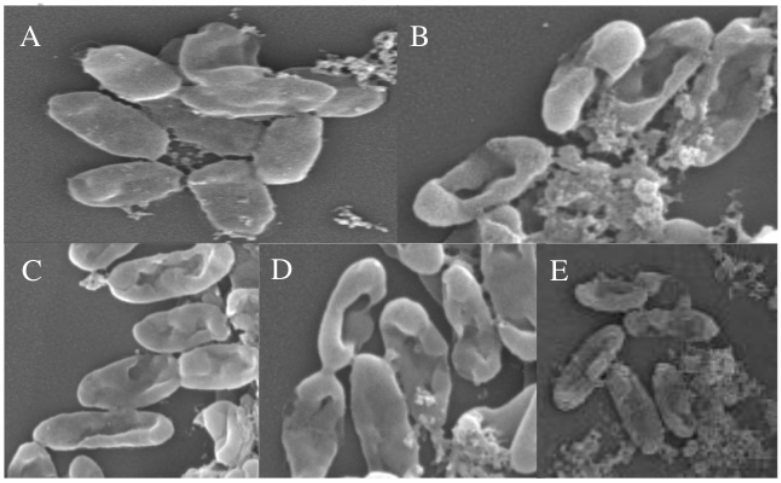


Figure 6. Scanning electron microscope photos. Morphological changes in *Klebsiella pneumonia* cells (A) Negative control (B) Positive control (Chloramphenicol) (C) treatment with Black Turmeric ethanol extract (D) Treatment with ethanol extract of Red Ginger (E) Treatment with ethanol extract of Mix.

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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