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Ethyl Acetate Extract of *Jatropha curcas* L. leaves as an Inhibitor of Histamine-Forming Bacterium in Fish Scombroid

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ABSTRACT

Jatropha curcas is known for its antimicrobial activity against various bacterial species, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Clostridium perfringens*. This study investigated the effects of ethyl acetate extract from *Jatropha curcas* leaves as an inhibitor of histamine-forming bacterium. The antibacterial activity was assessed by evaluating cell component leakage and observing morphological and structural changes in bacterial cells. Cell leakage induced by the extract was quantified using spectrophotometry at wavelengths of 260 and 280nm. The results demonstrate significant morphological and structural alterations in bacterial cells, as evidenced by the scanning electron microscopy (SEM). High absorbance values in the supernatant indicate substantial loss of nucleic acids, exceeding that of proteins. SEM analysis revealed disrupted cell division in *Klebsiella pneumoniae*, cell wall lysis in *Enterobacter aerogenes*, and abnormal cell wall structures in *Clostridium perfringens*.

INTRODUCTION

Various plant extracts and their antimicrobials have become the focus of research by researchers today and have been widely reported **(Cybulska *et al.*, 2016; Sitarek *et al.*, 2020; Yu *et al.*, 2021; Mustabi *et al.*, 2022)**. *Jatropha curcas* is a plant belonging to the Euphorbiaceae family found in Africa, Asia, and Latin America. This plant is traditionally used to treat microbial infectious diseases **(Burkill, 1985; Lewis, 1986)**, malaria in Mali **(Henning, 1997)** and for hemostatic treatment in Africa **(Gübitz *et al.*, 1999)**. It is also used for skin infections, diarrhea, as well as dysentery **(Mujumdar *et al.*, 2000)**. The development of bacteria that cause damage to fish can be inhibited in several ways, including adding compounds that have the potential to be bactericidal, as well as increasing histamine content in fish can be inhibited by adding natural extracts. One form of local wisdom in Maluku-Indonesia is that, the fishing community has utilized *J. curcas*

leaves to prevent histamine poisoning in scombroid fish by boiling the fish with *J. curcas* leaves. The formation of histamine in fish is greatly influenced by the concentration of histidine in fish tissue and the presence of bacteria that produce the HDC enzyme **(Visciano *et al.*, 2020; Mohamed *et al.*, 2023)**. Bacterial species such as *Morganella psychrotolerans, Photobacterium phosphoreum,* and several *Enterobacteriaceae* are significant histamine producers **(Yang *et al.*, 2020)**. Storage temperature and time also play a crucial role in histamine accumulation, with low temperatures reducing the risk of histamine formation **(Mohamed *et al.*, 2023)**. According to **Chen *et al.* (2010)**, histamine is formed from the decarboxylation of free histidine caused by several bacteria that have the HDC enzyme. The formation of histamine in fish is related to the concentration of histidine in fish tissue and the quantity and type of bacteria that produce histidine decarboxylase **(Satomi *et al.*, 2011)**. Bacteria from the genera *Photobacterium* and *Morganella* have a high capacity to produce histamine in fresh tuna during cold storage (2°C), and even under cold and vacuum packaging conditions **(Emborg *et al.*, 2005)**. **Guizani *et al.* (2005)** reported that at low temperatures (0°C), histamine formation is relatively slow, whereas at room temperature, histamine is produced in significant amounts within a short period, potentially posing a risk of poisoning.

The results of research by **Setha *et al.* (2014a)** reported that the bioactive components of *J. curcas* leaves contain alkaloids, phenolics, saponins, tannins, terpenoids. Furthermore **Lin *et al.* (2003)** stated that jatropin is an alkaloid substance found in the latex of the *J. curcas* plant which is suspected of having antitumor properties. Several other supporting studies that examined the potential of *J. curcas* to have antimicrobial activity **(Namuli *et al.*, 2011)**. Moreover, *J. curcas* also has antibacterial activity against *Staphylococcus aureus, Pseudomonas aeroginosa,* and *Escherichia coli* **(Aiyelaagbe *et al.*, 2007; Thomas & Sharma, 2008)**; the crude extract of *J. curcas* stems inhibits the growth of *Escherichia coli, Staphylococcus aureus,* and *Klebsiella pneumonia* bacteria **(Igbinosa *et al.*, 2009)**. Preliminary research by **Setha *et al.* (2014b)** indicates that the ethyl acetate extract of castor oil plant leaves is more effective in inhibiting the growth of histamine-forming bacteria in tuna (*Auxis thazard thazard*, L) stored at room temperature for 5 hours. However, so far the mechanism of action of ethyl acetate extract of *J. curcas* in inhibiting the activity of histamine-forming bacteria is unknown. Several types of bacteria play a role in the formation of histamine compounds in fish. According to **Sabry *et al.* (2019)**, *Enterobacter cloacae, Raoultella planticola, Citrobacter freundii* and *Enterobacter aerogenes* are the most frequent histamine-producing bacteria in fish. Fresh tuna is commonly contaminated with strong histamine-producing bacteria such as *Morganella psychrotolerans, Photobacterium phosphoreum, P. damselae,* and *Hafnia alvei* **(Trevisani *et al.*, 2019)**. Histamine-producing bacteria in fish include *Proteus vulgaris, P. mirabilis, Enterobacter aerogenes, E. cloacae, Serratia fonticola, S. liquefaciens, Citrobacter freundii,* and *C. braakii* **(Oktariani *et al.*, 2022)**. Enterobacteriaceae, particularly *Klebsiella pneumoniae*,

Staphylococcus xylosus, *Escherichia coli*, *Enterobacter cloacae*, and *Enterobacter aerogenes*, are histamine-forming bacteria in fish **(Sabry *et al*., 2019; Mahmoud *et al*., 2023)**. Based on this, this study was conducted to investigate the antibacterial mechanism of ethyl acetate extract of castor oil plant leaves against *Enterobacter aerogenes*, *Klebsiella pneumonia* and *Clostridium perfringens*. The selection of E*nterobacter aerogenes*, *Klebsiella pneumoniae*, and *Clostridium perfringens* as histamine-forming bacteria in the scombroid fish is based on their ability to produce histamine through the enzyme histidine decarboxylase, as well as their common presence in spoiled or contaminated fish. These bacteria are highly relevant in histamine research on seafood products because they can cause serious foodborne poisoning if fish are not handled or stored properly. Their growth is facilitated in conditions where temperature control is inadequate, emphasizing the importance of proper handling and refrigeration to prevent histamine accumulation and ensure food safety.

MATERIALS AND METHODS

Materials

The *Jatropha curcas* leaves were harvested from Waitatiri village, Ambon, Maluku Province, Indonesia. The organic solvents used were n-hexane, chloroform, hexane, ethyl acetate and methanol, filter paper, distilled water, nutrient agar (NA), nutrient broth (NB), buffer phosphate, buffer cocodylate, glutaraldehyde, and osmium tetraoxide. The bacteria used in this study were *Enterobacter aerogenes, Klebsiella pneumonia,* and *Clostridium perfringens* obtained from the Microbiology laboratory, Faculty of Medicine, University of Indonesia. The bacteria were maintained at 4°C on a nutrient Agar slant in the Microbiology laboratory, Faculty of Pharmacy, Hasanuddin University, Makassar Indonesia.

Equipment

Bacterial cell leakage determination was performed by using HERMLE Labnet Z383K centrifuge, analytical balance, vacuum rotary evaporator, petri dish, bottles, funnel, flask, Erlenmeyer, beaker glass, water bath, Whatman paper number 42, incubators, autoclave, Bunsen, stirrer-plate, micropipette, blender jar, UV-VIS spectrophotometer (Simadzu), shaker, Simadzu UV-VIS spectrophotometer. Determination of bacterial cell morphology changes was done by using JOEL 5310 scanning electron microscope (SEM).

Extraction

Jatropha curcas leaves powder was macerated in n-hexane and filtered then the residue was re-macerated in ethyl acetate. Maceration was done in 3 × 24 hours for each solvent. The filtrate was evaporated by using a rotary evaporator vacuum. The procedure

of determining cell leakage of histamine-forming bacteria affected by ethyl acetate extract of *J. curcas* leaves refers to **Setha *et al*. (2014a)**.

Research procedures

Determination of bacterial cell leakage by using a spectrophotometer (Bunduki *et al*, 1995)

Detection of bacterial cell components leakage affected by ethyl acetate extract of *J. curcas* leaves was identified by using a spectrophotometer at 260 and 280nm to determine nitrogen from bacterial nucleic acid and bacterial cells. Determination of bacterial cell leakage was done according to the following procedure: Pure culture bacterial suspensions were grown for 24 hours; 10ml were taken and put in a centrifuge tube and centrifuged at 3,500rpm for 20 minutes. The filtrate was removed, and the bacteria were washed by using 10ml phosphate buffer pH 7.0 and centrifuged again at 3,500rpm for 20 minutes (the washing process was performed 2 times). The bacterial cell obtained was treated in 10ml of phosphate buffer pH 7.0- and 0.2-mL ethyl acetate extract of *J. curcas* leaves with a concentration of 0 MIC (control), 1 MIC, and 2 MIC. After that, all tubes were shaken for 24 hours. The suspension was centrifuged at 3,500rpm for 20 minutes, and the filtrate absorbance was measured by using a spectrophotometer at 260 and 280nm.

Evaluation of bacterial cell damage by using a scanning electron microscope (Bozolla & Russel, 1999)

Bacterial cell damage was detected using a scanning electron microscope to learn the morphological and structural bacterial cell changes affected by ethyl acetate extract of *J. curcas* leaves. The changes in appearance, cell size, and the damage to the cell wall and cytoplasmic membrane were determined with the following assay procedure: Pure cell culture suspension aged 24 hours treated by using ethyl acetate extract of *J. curcas* leaves at a concentration of 1% and centrifuged at 3500rpm for 15 minutes. The specimen was washed 2 times by using a phosphate buffer solution. The precipitated bacterial cell was obtained after being centrifuged and treated by using 2.5% glutaraldehyde (pH 7.3) and left to stand for 2 hours. After that fixed by using 1% osmium tetroxide in a 0.05% cocodylate buffer (pH 7.2) for 2 hours, then washed by using aquabides (DDH2O) 3 times, for 2 minutes each. The specimen was then washed by using phosphate buffer solution 2 times, after the solution was discarded, added gradient series of ethanol (25, 50, 75, and 100%) were added 3 times for 10 minutes each. Afterward, each sample was glued to aluminum stubs and then coated with gold through a vacuum process (6-7pa) for 20 minutes and observed by using SEM JOEL 5310.

Data analysis

Data were analyzed descriptively to reveal cell leakage and damage to bacterial cells.

RESULTS AND DISCUSSION

1. Bacterial cell leakage

Spectrophotometer absorbance observation showed the cell supernatant absorbance increasing at 260 and 280 nm. The loss of nucleic acid components from the cytoplasmic membrane due to bacterial cell leakage is indicated by absorbance values around 0.000-0.796 at 260nm (Fig. 1). The loss of protein components from the cytoplasmic membrane, indicated by absorbance values ranging from 0.000 to 0.734 at 280nm (Fig. 2), suggests bacterial cell component leakage.

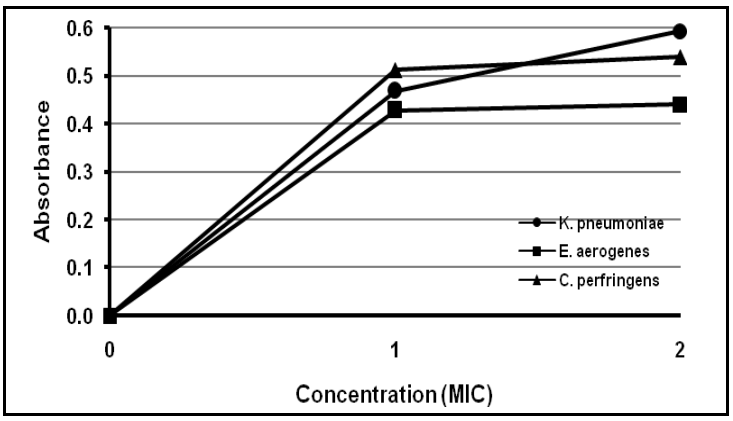

Fig. 1. Graph of bacterial cell leakage measured at 260nm

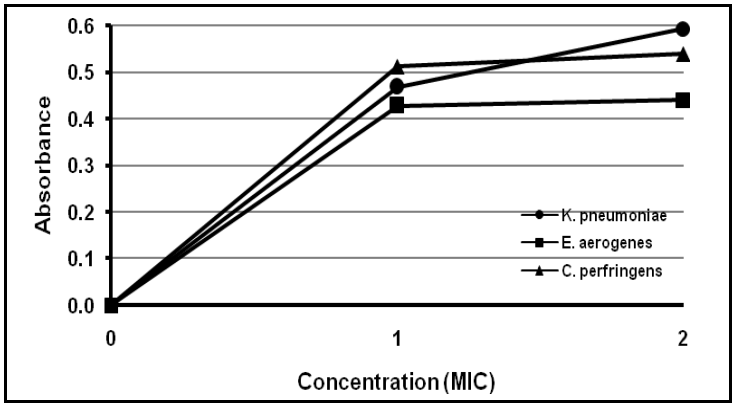

Fig. 2. Graph of bacterial cell leakage measured at 280nm

An absorbance increasing value indicates the increasing amount of nitrogen compounds flowing out from the cytoplasmic membrane of the bacterial cell (Figs. 1, 2). Absorbance compounds at 260nm are RNA and its nucleotide derivates, while absorbance compounds at 280nm are proteins **(Ranjbar & Gill, 2009)**.

Spectrophotometer observation at 260nm detects purines, pyrimidines, and ribonucleotides, while at 280nm detects tyrosine and tryptophan **(Park *et al.*, 2003)**. Releasing nucleic acids and proteins indicates cell leakage due to cytoplasmic membrane damage or cell membrane permeability changes. Microbial inhibition by antimicrobial compounds commonly through 4 mechanisms, 1) interference with the components of the cell, especially the components of the cell wall, 2) reaction with cell membranes and resulting permeability changes and inducing loss of cell constituent components, 3) inhibition of protein synthesis and 4) impaired function of genetic material **(Davidson *et al.*, 2012)**. Antimicrobial compounds attack cytoplasmic membrane and affect membrane integrity leads cell membrane permeability enhancing and letting antibacterial components penetrate the cells **(Xu *et al.*, 2017; Babii *et al.*, 2018)**. Cytoplasmic membrane damage enhances permeability and lead cell leakage followed by intracellular material release **(Davidson *et al.*, 2012)**. In this study, the absorbance value at 260nm is greater than 280nm indicating leakage of nucleic acid compounds (RNA and its derivates) greater than proteins leakage. The leakage of bacterial membrane proteins is a critical phenomenon that may result from various mechanisms, including the activity of antimicrobial peptides. The F1 antimicrobial peptide from Tibetan kefir disrupts the membrane structure of both Gram-negative and Gram-positive bacteria, causing cell death by inducing leakage of cell contents and damaging the phospholipid membrane **(Wang *et al.*, 2021)**.

The relationship between increased absorbance and bacterial cell component leakage can be explained through the mechanism of membrane damage. The bacterial cytoplasmic membrane is the primary barrier that separates intracellular components from the external environment. Under normal conditions, this membrane maintains osmotic balance and prevents the leakage of essential components, such as nucleic acids and proteins. However, when the membrane is damaged, it loses its integrity. The leakage of nucleic acids and proteins from bacterial cells is typically an early sign. This indicates irreversible membrane damage. The observed increase in absorbance at wavelengths of 260 and 280nm indicates that these essential components have leaked, signifying substantial cell damage. This leakage can lead to cell death, as nucleic acids, essential for replication, and proteins, vital for enzymatic functions, are lost from the cell. The results showing leakage of nucleic acids and proteins suggest important implications for developing new antimicrobial agents. Antimicrobial agents designed to target the bacterial cell membrane can effectively kill bacteria by inducing the leakage of vital components from the cell. This strategy is considered highly effective in combating pathogenic bacteria that are resistant to conventional antibiotics.

Clostridium perfringens has the greatest cell leakage at a concentration of 1% MIC, while *Enterobacter aerogenes* has the smallest cell leakage. *C. perfringens* was more sensitive to ethyl acetate extract of *J. curcas* leaves with a MIC value of 0.10% than *E. aerogenes* with a MIC value of 0.75%. At a concentration of 2% MIC, *K. pneumoniae*

exhibited greater cell leakage than *C. perfringens* and *E. aerogenes*. *C. perfringens* is a Gram-positive bacterium with a single, thick-layered cell wall structure (12-80 µm) composed primarily of peptidoglycan, with less lipid and the presence of polysaccharides (teichoic acid). Teichoic acid is a water-soluble polymer that facilitates the transport of positive ions into and out of the cell. The water-soluble compounds in Gram-positive bacteria indicate a more polar nature, allowing the cell wall of *C. perfringens* to be easily penetrated by the semipolar secondary metabolite compounds in the ethyl acetate extract of *J. curcas* leaves.

The antimicrobial mechanism of the ethyl acetate extract against *C. perfringens* involves penetration into the peptidoglycan layer and binding to proteins. It is assumed that bacterial cell damage depends on the type of secondary metabolite present in the ethyl acetate extract.

Enterobacter aerogenes, a Gram-negative bacterium, has a complex cell wall structure consisting of an outer lipoprotein layer, a middle layer of lipopolysaccharide that serves as a barrier to bioactive antibacterial agents, and an inner peptidoglycan layer containing 11-22% lipid **(Jawetz *et al.*, 2005)**. The lipid-rich Gram-negative cell wall provides a strong barrier against semipolar and polar secondary metabolite compounds found in the ethyl acetate and methanol extracts of *J. curcas* leaves. Therefore, Gram-negative bacteria are generally more resistant to the antibacterial compounds in these extracts.

The antibacterial mechanism of the ethyl acetate extract against *E. aerogenes* involves a reaction with purine proteins on the outer membrane, followed by penetration into the peptidoglycan layer. As with *C. perfringens*, the damage to bacterial cells in *E. aerogenes* is influenced by the type of secondary metabolite present in the extract.

Bacterial cell damage

Klebsiella pneumoniae is a Gram-negative bacterium, rod-shaped, measuring 0.5-1.5 × 1-2µm, non-spherical, non-motile, capsule forming both invivo and invitro, the colonies are slimy (mucoid), lactose-fermenting and facultative anaerobic (Fig. 3a). *K. pneumoniae* showed cell elongation and division due to being affected by the ethyl acetate extract of *J. curcas* leaves with a concentration of 1% (arrow on Fig. 3b). Cell division should be divided into two with the same size and properties as stem cells. The cell shape shown in Fig. (3b) with septa has already formed, but the cell has not yet divided. When the process of septa division is disrupted, bacterial cell growth will be inhibited. The disruption of the cell division process is affected by the ethyl acetate extract of *J. curcas* leaves. Phytochemical test results showed the ethyl acetate extract of *J. curcas* leaves contains alkaloids, phenolic, tannins, terpenoids, and saponins **(Setha *et al.*, 2014b)**. The mechanism of ethyl acetate extract of *J. curcas* leaves

against *E. aerogenes* is suspected by reacting with porin protein on the outer membrane of bacterial cell walls, then entering the peptidoglycan layer. Bacterial cell damages depend on the type of secondary metabolite compounds in the ethyl acetate extract of *J. curcas* leaves. One of the mechanisms antimicrobial compounds is interfering with the formation of nucleic acids (DNA and RNA) and influencing the genetic information transfer that inhibits the activity of RNA polymerase and DNA polymerase enzymes **(Mariani & Maffioli, 2009)**. The further mechanism to destroy genetic material has bad implications for the cell division process for propagation. Flavonoid compounds play a role in the inhibition of DNA-RNA synthesis by intercalation or buildup hydrogen bonding with nucleic acid base **(Cushnie & Lamb, 2005)**, it can be thought that the phenolic compounds in *J. curcas* leaves extract damaging genetic material and disrupts the process of cell division for propagation.

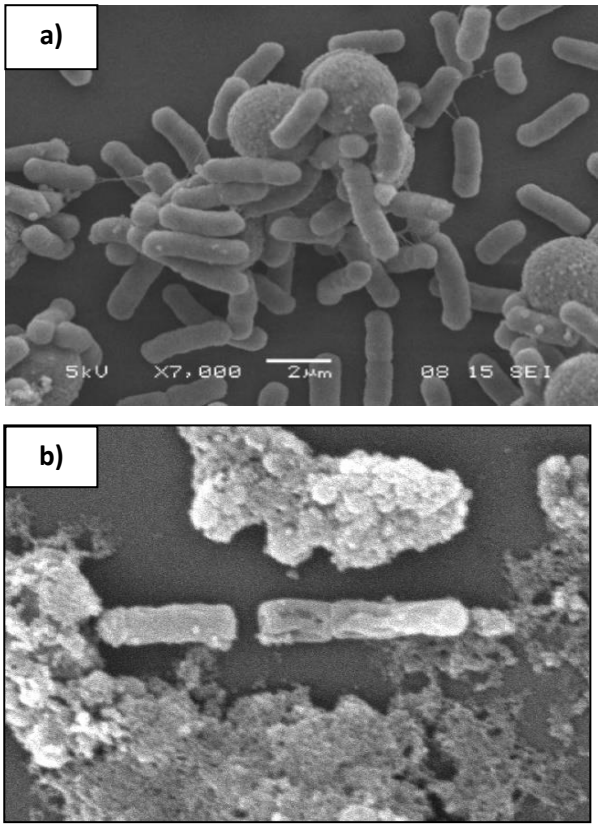

Fig. 3. a) Normal cell *K. pneumoniae* bacteria, magnification of 7.000 ×; **b.** Morphological changes in bacterial cells of *K. pneumoniae* affected by ethyl acetate extract at a concentration of 1%, magnification of 10.000x

Enterobacter aerogenes is a Gram-negative bacterium, facultatively anaerobic, rod-shaped, diameter 0.6-1.0µm, length 1.2-3.0µm, flagella, motility (can move), smooth form colonies and optimum growth temperature of 37 ^{o}C **(Patadia, 2020)**. The shape of *E. aerogenes* normal cell bacteria can be seen in Fig. (4a). Cell wall lysis resulting

morphological changes of *E. aerogenes* due to being affected by ethyl acetate extract of *J. curcas* leaves with a concentration of 1%. *E. aerogenes* with cell walls are lattice turning darker/blackish (arrow on Fig. 4b). The damage of *E. aerogenes* cell wall can be seen by the appearance of protrusions and waves on the cell wall affected by secondary metabolites of alkaloids, phenolics, tannins, terpenoids, and saponins in the ethyl acetate extract of *J. curcas* leaves. The appearance of a bulge in the cell wall is caused by the porosity increase of the cell wall membrane (space) due to the weakening of the cell wall affected by secondary metabolite compounds on ethyl acetate extract of *J. curcas* leaves. Porosity increasing causes cell membrane permeability changes and leads cell leakage. Changes in cell wall permeability cause cytoplasmic fluid to seep out and form a space between the cytoplasmic membranes. This space gets bigger along with the weakening of the cell wall. The state of the membrane cannot withstand the pressure of the cytoplasm, which leads to membrane leakage followed by cytoplasm flowing out from the cell. A large amount of cytoplasmic comes out causing cell contraction and death. The mechanism of the ethyl acetate extract of *J. curcas* leaves activity against *E. aerogenes* is suspected by reacting with porin protein on the outer membrane of a bacterial cell wall, then entering the peptidoglycan layer. The bacterial cell damage depends on the type of secondary metabolite compounds of *J. curcas* leaves extract. The antibacterial mechanism of alkaloids is suspected by disrupting the peptidoglycan components in the bacterial cell wall consequently, the cell wall layer is not completely formed and causes cell death. Alkaloid compounds are basic groups that contain nitrogen and can react with the amino acids that make up the cell wall and bacterial DNA **(Harborne, 2006)**. This reaction alters the structure and composition of amino acids, leading to changes in the genetic balance within the DNA chain. These changes can promote lysis, potentially resulting in bacterial cell death.

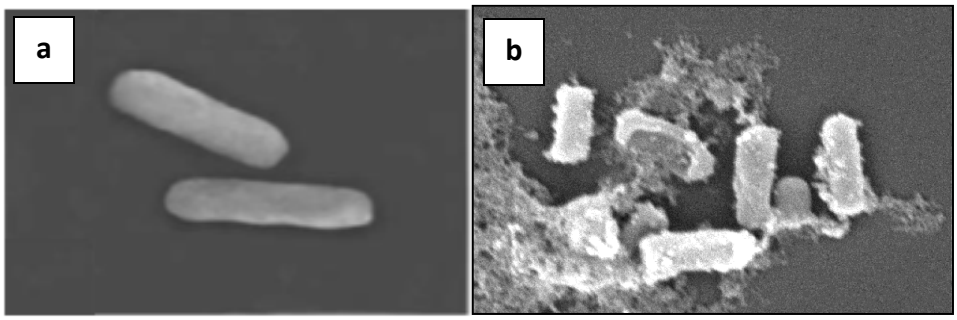

Fig 4. a). Normal cell *E. aerogenes* bacteria, magnification of 15.000 ×; **b)** Changes in bacterial cell morphology of *E. aerogenes* affected by ethyl acetate extract at a concentration of 1%, magnification of 10.000×

Phenolic compounds and their derivatives (tannins) are antibacterial working by damaging the cytoplasmic membrane. At low concentrations, it can damage the cytoplasmic membrane which causes leakage of important metabolites that activate the bacterial enzyme system, while at high concentrations it can damage the cytoplasmic membrane and precipitate cell proteins. Upon the destruction of the cytoplasmic membrane, H^+ ions from phenol compounds and their derivatives will attack the polar group (phosphate group) so that the phospholipid molecule will break down into glycerol, carboxylic acid, and phosphoric acid. This causes the cytoplasmic membrane to leak, and bacteria will experience growth retardation and even death **(Shahryari *et al.*, 2018)**. Terpenoids and their derivatives exhibit potent antibacterial activity, with several mechanisms involving increased membrane permeability and decreased membrane integrity **(Mahizan *et al*., 2019; Ergüden 2021; Bouyahya *et al*., 2022)**. The mechanism of terpenoids as an antibacterial is thought to damage the cytoplasmic membrane lipid fraction, and even the lysis of bacterial walls so that the lipid fraction is unable to maintain the cytoplasmic membrane shape **(Harborne, 2006)**. As a result, the cytoplasmic membrane will leak, and bacteria will experience growth retardation and even death. Antimicrobial compounds can react with the phospholipid component of the cell membrane, which results in cell lysis **(Ryan & Ray, 2004)**. Cell wall lysis can cause the cell wall to be separated all or in part, in Gram-negative bacteria called spheroplasts and in Gram-positive bacteria called protoplasts.

Clostridium perfringens is a Gram-positive rod-shape bacteria with a length of 4-8 and width of 0.8-1.5µm, facultatively anaerobic and able to produce H_2 gas, nonmotile, encapsulating and forming spores **(Ryan & Ray, 2004)**. The normal shape *C. perfringens* cell can be seen in Fig. (5a). Indentation on bacterial cell wall due to being affected by ethyl acetate extract of *Jatropha curcas* leaves at a concentration of 1% results in cell death (arrow on Fig. 5b). The formation of indentations in the cell wall is caused by leakage of the cytoplasmic membrane of the cell, where fluid from inside the cytoplasm comes out and causes the cells to contract. The effect of secondary metabolites of alkaloid, phenolic, tannin, terpenoids, and saponins contained in the ethyl acetate

extract of *J. curcas* leaves against *C. perfringens* can cause bacterial cell walls to become bumpy and there are protrusions caused by the porosity of cell membranes due to weakening of cell walls by the effect of secondary metabolite compounds in ethyl acetate extract of *Jatropha curcas* leaves. Changes in cell wall permeability cause cytoplasmic fluid to seep out so that space is formed between the cytoplasmic membrane. This space will get bigger with the weakening of the cell wall. In the state of the membrane that cannot withstand the pressure from the cytoplasm, the membrane leaks, and the cytoplasmic fluid flow occurs out of cells in large numbers, causing cells to contract (shown by the arrow in Fig. 5b). The mechanism of action of secondary metabolite compounds from ethyl acetate extract of *J. curcas* leaves against *C. perfringens* is suspected by entering the peptidoglycan layer and then binding to proteins. Furthermore, the damage that occurs in bacterial cells depends on the type of secondary metabolite compounds found in *J. curcas* leaves extract. The secondary metabolite compounds contained in the ethyl acetate extract of *J. curcas* leaves have 3 main compounds namely alkaloids, phenolics, and terpenoids. The mechanism of action of secondary metabolites of alkaloids, phenolics, and terpenoids as antibacterials can cause lysis **(Guleria *et al.*, 2023, 2024)**.

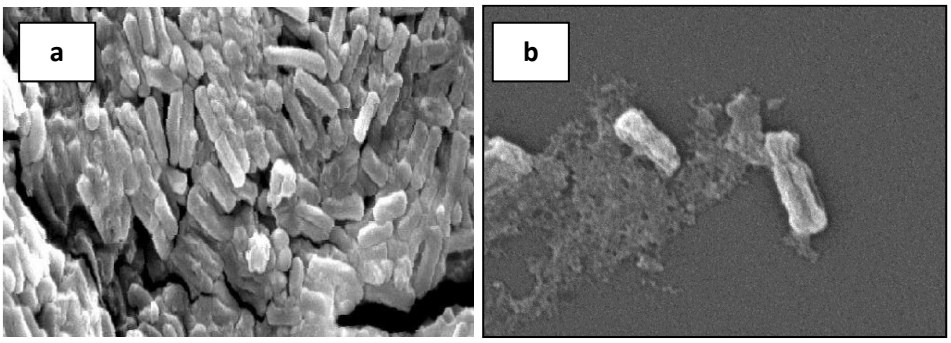

Fig. 5a. Normal cell *C. perfringens*, magnification of 5.000 ×; **b)** Morphological changes in *C. perfringens* bacterial cells affected by ethyl acetate extract at a concentration of 1%, magnification of 10.000×

CONCLUSION

Based on the results of the study, the leakage of bacterial cell components was indicated by the loss of nucleic acid components greater than the loss of protein components. In addition, the results of the scanning electron microscopy showed a disruption of *Klebsiella pneumoniae* cell division, lysis of the bacterial cell wall of *Enterobacter aerogenes*, and abnormalities (dentations) in the cell wall of *Clostridium perfringens*.

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