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Test of the Bacteriological Ability of Mangrove Nyirih (*Xylocarpus granatum*) as a Water Disinfectant

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ABSTRACT

Coliform bacteria contamination in wetlands poses a risk of causing waterborne diseases. Disinfectants are bactericidal, effectively reducing bacterial colonization in water. The coastal plant of mangrove nyirih or *Xylocarpus granatum* (*X.granatum*) contains various antibacterial compounds, so it can be used as a natural disinfectant. This study aims to analyze the bacteriological ability of mangrove nyirih as a disinfectant in water samples contaminated with Coliform. This laboratory experimental study used a pretest-posttest control group design. The dilution method was used to observe the effects of 25%, 50%, 75%, and 100% ethanol extract of mangrove leaves (L) and bark (B) of *X.granatum* as well as chlorine control. The parameters observed were the Coliform Most Probable Number (MPN) and Total Plate Count (TPC) in the test water sample. The results of the study, obtained a decrease in the Coliform MPN and TPC numbers of water samples after treatment. LB100% *X.granatum* extract produces an effect that is not significantly different from chlorine. In conclusion, mangrove nyirih has the ability as a water disinfectant.

Keywords: Coliform, Disinfectant, MPN, TPC, Water, *Xylocarpus granatum*

1. Introduction

Water quality in several regions in Indonesia, including South Kalimantan Province, is a common health problem due to natural and anthropogenic factors (Farihatini, 2019). Waterborne diseases such as diarrhea and gastroenteritis are closely associated with unhygienic behavior. Infectious diseases can be caused by the use of river water sources or piped water (PDAM) that are contaminated with Coliform bacteria or *Escherichia coli (E.coli*) (Budiarti et al., 2017). *Escherichia coli* is a gram-negative coliform bacteria, which is used as a biological indicator of water contamination by coliform feces of humans and warm-blooded animals. The bacteriological analysis to determine the antibacterial activity against Coliform contamination is the Most Probable Number (MPN) Coliform and Total Plate Count (TPC) methods. The results of the examination of samples of piped water used by communities around riverbanks and in the temporary disposal area (TPS) in Banjarmasin City, South Kalimantan are known to have a fairly high MPN Coliform index value (Heriyani et al., 2020; Heriyani et al., 2021).

A common effort made by the community to reduce the number of microbial contaminants in water sources (wells, reservoirs) is by adding a disinfectant substance such as chlorine at a concentration of 2 ppm (0.0002%). Chlorine is an effective and easy-to-use disinfectant. According to Sofyan (2018), Chlorine has a mechanism that destroys the structure of bacterial cells so that Coliform bacteria will die. According to Herawati and Yuntarso (2018), the effects of using chlorine in the long term can cause itching on the skin.

According to Safitri et al. (2020), the use of disinfectant preparations can also be obtained naturally from plants that have antibacterial activity. Laboratory test results and treatment of mangrove api-api extract or *Avicennia marina (A.marina),* can reduce the MPN number of pathogenic *E.coli* contamination in marine fish Euthynus affinis. According to Giri et al. (2011), 75% of mangrove plants are widespread in 15 tropical and subtropical countries and 22.6% in Indonesia. Mangrove forest ecosystems not only play an important role in the ecology of the coastal and coastal areas of the Indonesian archipelago but also empirically used in medicine (Purwanti et al., 2016).

This type of mangrove nyirih or *Xylocarpus granatum (X.granatum)* is one that is often found in coastal areas. This type is efficacious as a medicine as a medicine for itching and diarrhea. According to Nasution et al. (2020), the pharmacological and biological effects of *X.granatum* can be used as a medicine for cholera, dysentery, fever, antibiotics, as well as an antiseptic agent. According to Dey et al. (2021) and Mardiansyah et al. (2016), pharmacological and biological effects of *X.granatum* activity can inhibit gram-positive and gram-negative pathogenic bacteria in fish. Its also has an effect on human pathogenic bacteria (Batubara, et al., 2021; Yoswati et al., 2021). According to Shaheb et al. (2016), the ingredients that act as bioactive in this plant include saponins, flavonoids, enols, alkaloids, tannins, and steroids. The content of secondary compounds in *X.granatum* was reported to be more found in the leaves and bark. Its inhibitory activity against *E.coli* from bark extract is greater than leaf extract at the same concentration (Hendrawan et al., 2015).

According to Budiarti et al. (2022), research on *X.grantum* extract on a single preparation showed antibacterial activity under the effect of positive control. Administration of a mixed extract of *X.granatum* bark leaves from the mangrove forest of Pulau Burung, South Kalimantan, can have an effect equivalent to that of an alcohol antiseptic. This study aims to analyze the bacteriological ability of mangrove nyirih (*Xylocarpus granatum*) as a disinfectant in test water samples modified with Coliform bacteria. This research was approved by the ethical commission of the Faculty of Medicine, University of Lambung Mangkurat (ULM), with letter number: 205/KEPK-FK ULM/EC/VII/2022.

2. Materials and Methods

Materials

This research was conducted at the Laboratory of Microbiology and Pharmacology, Faculty of Medicine, University of Lambung Mangkurat, Banjarbaru. carried out in September-December 2022. The test plant for the *Xylocarpus granatum* type was obtained with permission from the South Kalimantan Natural Resources Conservation Agency (BKSDA), originating from Bird Island in Batulicin, Tanah Bumbu Regency, South Kalimantan.

This laboratory experimental study used a pretest-posttest control group design. The test treatments included the combined ethanol extract of the leaves and bark of mangrove nyirih in 5 treatment groups, a positive control with 0.0002% chlorine solution, and a negative control with 1% DMSO. Repetition of treatment 3 times obtained from Federer's calculations. The observation method was the Coliform Most Probable Number (MPN) and Total Plate Count (TPC) before and after treatment. Sample tests and observation of MPN and TPC values were carried out three times.

Methods

Extracts were prepared from each sample of *X.granatum* bark and leaves, namely as much as 1 kg; samples were washed and ground into simplicial. The simplicia was extracted one by one with 96% ethanol, for 72 hours at room temperature and occasionally stirred with a glass rod. After 3 days, the mixture is filtered using Whatman filter paper no.1. The extract obtained was dried and concentrated in a rotary vacuum evaporator, at 40°C. About 100 grams of each extraction was stored in the refrigerator at 4°C for further analysis. In the test extract concentration of 100% (w/v), serial dilutions of the extract were made with 1% DMSO solvent, so that concentrations of 75%, 50%, 25%, and 12.5% were obtained. Extract combinations were made by mixing each concentration of the test extract at a ratio of 1:1 (Budiarti, Rizky, 2021).

The test water sample used in this study was a sample of piped water (PDAM) in the Microbiology Laboratory of FK ULM Banjarbaru. A suspension of coliform bacteria/*E.coli* ATCC 25923 was prepared in Broth Infusion Heart (BHI) Scharlow media equivalent to Mac Farland 0,5. Testing water samples using the Most Probable Number (MPN) method refers to the method of Dewi & Putriani (2022). The MPN coliform test includes 1). Presumption test, observing the growth of Coliform bacteria in Lactose

Broth (LB) Scharlow media. 2). Coliform positive confirmation test was performed on Brilliant Green Lactose Broth (BGLB) Scharlow media. The results of incubation for 24-48 hours (37°C), the test tube was positive which showed turbidity and gas in the Durham tube. The results of the MPN test are the Coliform MPN values/index in 100 ml compared to the 5-1-1 variant MPN test table.

Test the Total Plate Number (TPC) in water samples using the 10^{-1} to 10^{-3} dilution method. The TPC test refers to the method of Ariani et al. (2018) with modifications. The dilution of the extract in the test tube used 1% DMSO solution, while the chlorine dilution used 0.9% physiological solution. Sequentially, each test water sample in the test tube was added to the treatment. Next, 1 ml of suspension was taken from each tube to be put into a Petri dish and added to Nutrien Agar (Agar Plate Count). Counted bacterial colonies that grew after an incubation period of 2 x 24 hours (37°C). Count the number of growing colonies. MPN and TPC pretest tests were carried out on water samples modified by *E. coli* equivalent to Mac Farlan 0.5.

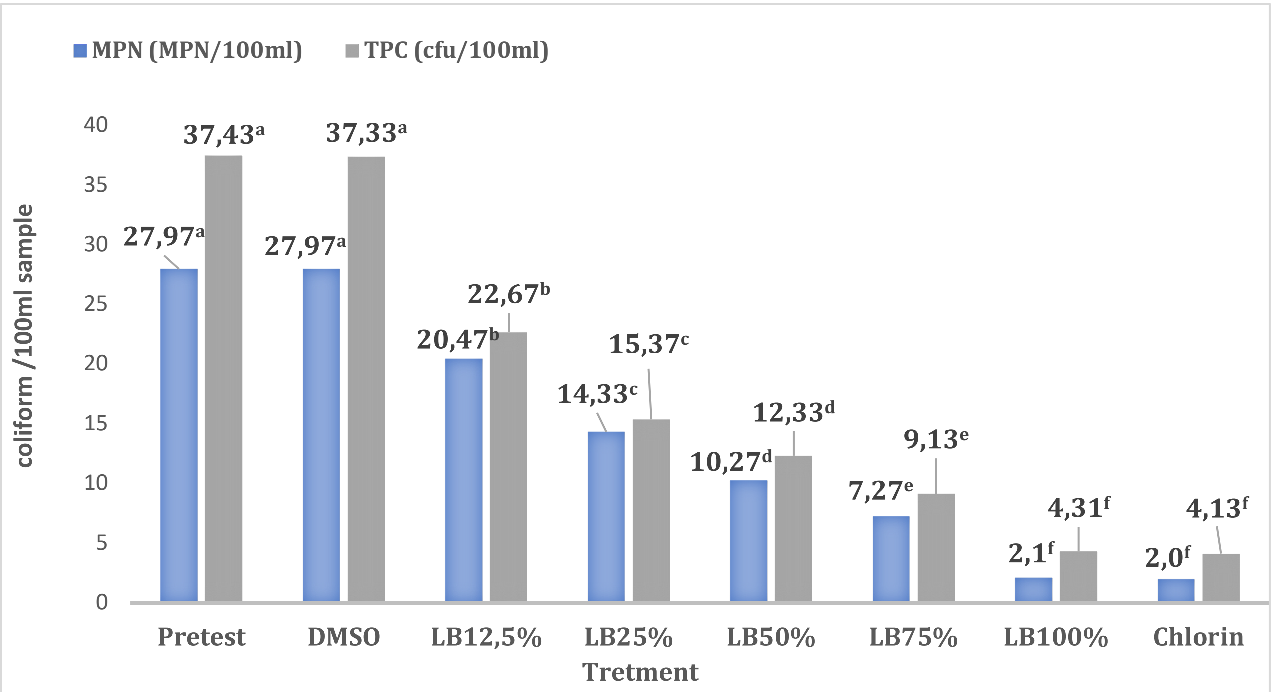
The phytochemical test of the extract was carried out according to Harborne's standard (1987) with modifications. The tests included Wagner and Dragendroff tests for alkaloids; alkaline tests and lead acetate tests for tannins; tests for flavonoids, phenolics, steroids, terpenoids, and foam tests for saponins (Pramiastuti et al., 2020).

Statistical analysis of research data used IBM SPSS Statistics 26 for Windows, at a 95% confidence level. Test the normality and homogeneity of the data using the Shapiro-Wilk test and Levene's test. Analysis of whether there was a difference in effect and comparison of the effects of each treatment used the Kruskal Wallis test and Wilcoxon posthoc test.

3. Results and Discussion

MPN and TPC Coliform Values of Test Water Samples

In this study, the MPN Coliform index value before treatment was 27,97 MPN/100ml and the average TPC value before treatment was 37,43 cfu/100ml. The observed results of the average MPN index values and the average TPC values of the test water samples and the results of statistical analysis are shown in Figure 1.



Description: LB = combination extract of leaves and bark. The same letter above the line indicates that the treatment did not have a different effect based on the Wilcoxon test at the 5% real level.

Figure 1. The average MPN and TPC Coliform values (CFU/100ml) of test water samples before and after treatment

The mean MPN and TPC Coliform values in the pre-test water samples were not significantly different from the 1% DMSO negative control. That is, DMSO as a solvent extract does not produce antibacterial effects. Post-test results, administration of a combination of leaf and stem bark extract (LB) of *X.granatum* concentration of 12.5% - 100% and chlorine was able to reduce the number of bacteria. Treatment of 100% LB extract produces antibacterial power equivalent to chlorine. Chlorine as a disinfectant has been tested for its effectiveness. Disinfection with chlorine causes changes in the permeability of the *E.coli* membrane because chlorine oxidizes amino acids phospholipids and peptidoglycan (Xu et al., 2018). Phospholipids are a component of the plasma membrane, while peptidoglycan is a component of the cell wall.

Increasing the concentration of LB extract affects its activity in reducing the colonization of *E. coli* bacteria. Increasing the concentration can increase the solubility and stability of the extract's bioactive compounds, thereby increasing its effectiveness as an antibacterial. The effectiveness of the 100% *X.granatum* extract which has the most effect on reducing the number of Coliform in this study, is relatively the same as the test results of Budiarti et al. (2022) as an antiseptic with 70% alcohol control. This shows that the mangrove extract does not only have an antiseptic effect but also acts as a water disinfectant.

Coliform MPN values according to WHO (2011): a) Low risk: 0-10 MPN/ 100 ml of water sample, b) Intermediate risk: 10-100 MPN/100 ml of water sample, c) High risk: 100-1000 MPN/100 ml of water sample (WHO, 2011). Based on Permenkes No. 492 of 2010, the number of bacteria allowed in PDAM water is 10 in 100 ml of piped water samples and 50 in 100 ml of non-piped water samples. Permenkes Number 32 of 2017 for water quality standards, the maximum total Coliform for sanitary hygiene purposes is 50 MPN/100 ml (Permenkes, 2010; 2017). Administration of a combination extract of *X.granatum* LB 75% and LB 100% showed the MPN values of water samples in the low-risk category, namely 7.27 MPN/100ml and 2.1MPN/100ml. The TPC values of the 75% LB and 100% LB extracts also produced the number of bacteria according to the Minister of Health, namely 9.13 cfu/100ml and 4.31 cfu/100ml. However, the 100% LB extract produced better coliform-killing effectiveness.

The synergistic effect of antibacterial compounds in the combination of LB extracts is able to work effectively to inhibit Coliform colonization in water samples. The results of the phytochemical screening showed the same secondary compound content in the leaves and bark of *X.granatum*. Among them are alkaloids, flavonoids, phenols, tannins, terpenoids, and steroids. The phytochemical content in this study was relatively different from the results of Shaheb et al (2016); where saponin compounds were not found in the ethanol extract of stem bark. The content of secondary compounds in the extract is responsible for its activity as an antibacterial, but this is affected by the dose of the extract. The synergistic effect of antibacterial compounds in the combination of LB extracts is able to work effectively to inhibit Coliform colonization in water samples. Polyphenols and flavonoids are among those that work synergistically (Ding et al., 2022); as well as tannin compounds (Carvalho, et al., 2018).

The mechanism of action of alkaloid compounds has an inhibitory mechanism by interfering with the constituent components of peptidoglycan in bacterial cells so that the cell wall layer does not form completely and causes cell death. Flavonoids, saponins, and tannins belong to the class of phenolic compounds (Yan et al., 2021). Flavonoids can damage cell membrane function, as well as inhibit DNA-RNA synthesis and energy metabolism (Syarif et al., 2014). According to Khalid et al. (2019), the action of tannin by forming hydrophobic complexes with proteins, enzymes, and cell wall transport proteins, thereby interfering with bacterial growth. Additionally, tannins can shrink cell walls or cell membranes thus they interfere with the permeability of these cells, Cells cannot carry out living activities, inhibit growth, or even die (Hossain et al., 2021). Saponin compounds are antibacterial, work by diffusion through the outer membrane and vulnerable cell walls, then bind to the cytoplasmic membrane thereby disrupting and reducing membrane stability, ultimately causing cell death due to leakage of cytoplasm from the cell (Yu-Pu & Pi-Hu, 2020). Terpenoid compounds can react with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall and form strong polymeric bonds that damage the porins (Broniatowski & Mastalerz, 2015). Damage to the porin, which is the entry and exit point for compounds needed by the bacterial cell, will reduce the permeability of the bacterial cell wall so that the bacterial cell lacks nutrients so that the growth of the bacteria is inhibited or dies (Carvalho et al., 2018).

In circumstances where the secondary compounds work synergistically in inhibiting *E. coli,* the extract lowers the MPN and TPC Coliform values in the water samples. This makes the inhibitory activity of 100% LB extract equivalent to that of chlorine disinfectants. Based on this research, the extract of mangrove *X.granatum* has the potential as a natural disinfectant. Proving its effectiveness as a disinfectant, it can be assessed based on the phenol coefficient test.

4. Conclusions

Administration of a combination extract of *X.granatum* 75% and 100% was able to inhibit bacterial contamination in water samples contaminated with *E. coli* bacteria. The extract was able to reduce the MPN and TPC Coliform values of water samples to meet health standards. The results of this study prove that the extract of the mangrove nyirih has potential as a water disinfection agent.

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Conflict of Interest

Researchers in this team do not have a conflict of interest with any party.

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