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Ability Test of Taro (*Colocasia esculenta* L.) Leaves Extract to Reduce Coliform Bacteria in River Water

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Abstract - The problem of coliform bacteria pollution in river water can be overcome by water chlorination. However, chlorine in water can cause toxic side effects. The use of phytochemicals can be an alternative disinfectant. Taro or *Colocasia esculenta (C. esculenta)* is a functional food plant and contains antibacterials. This study aims to analyze the ability of *C. esculenta* leaf extract to inhibit the colonization of Coliform bacteria in river water samples. The laboratory research method uses a pre- and post-design with a control method to assess the bacteriological quality of water before and after extract treatment (concentration 7,5mg/L - 90mg/L and chlorine control). The parameters assessed were MPN (Most Probable Number) and Total Plate Count (TPC) Coliform. In qualitative phytochemical screening, the bioactive content of phenol, flavonoid, saponin, tannin, steroid, and terpenoid compounds was identified. The results showed the ability of *C. esculenta* leaf extract to reduce the number of MPN and TPC coliform in water samples; significant extract concentrations at 60 mg/L and 90 mg/L. Anti-Coliform activity is produced by compounds dissolved in the extract. Conclusion, shows the potential of *C. esculenta* leaf extract as a natural disinfectant in reducing the colonization of aquatic Coliform.

Keywords: *Colocasia esculenta*, Coliform, leaves extract, river water

1 Introduction

Water is a major natural resource that meets human needs as a very valuable asset [1]. The earth's surface is about 71% water, but only 1% of the total water reserves can be used for needs and activities. The availability of river water that has been contaminated with microbes is not suitable for direct use [2]. In addition, climate change and land use patterns cause depletion of groundwater resources and reduce water quality. In addition, climate change and land use patterns cause depletion of groundwater resources and reduce water quality. This results in about 50% of the population in developing countries suffering from digestive tract diseases caused by unhygienic water sources [1]. According to the World Health Organization (WHO), the use of unsafe water has an impact on more than 3.4 million child deaths worldwide/year [3].

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The quality of river water in Indonesia, as in other developing countries, is in a severely polluted state. Environmental health data in 2015-2016, reported that out of 471 monitored river points, 343 river points showed poor quality due to microbial contamination [4]. The impacts of contamination that are often found in the community are symptoms of digestive tract infections and diarrhea. In Indonesia, diarrhea is an endemic disease that has the potential to become an extraordinary event causing death in children. Data from the South Kalimantan Provincial Health Office in 2023, reported that there were 2,193 cases out of 59,398 total visits of infectious disease sufferers, and the highest cases were diarrhea (1,109 cases) [5].

The bacterial agents that cause diarrhea that are often found are Coliform or *Escherichia coli* (*E. coli*). Coliform is used as a biological indicator of contamination in water.[6] According to the Ministry of Health of the Republic of Indonesia (2017), the total number of coliform bacteria permitted in water samples is 50 CFU/100 ml. However, in general, waters in South Kalimantan have coliform contamination quality that exceeds health limits [7–9]. This causes diarrhea cases to often relapse and cause carriers. Factors that influence it are the availability of clean water and healthy toilets, inadequate sanitation in households, and unhygienic human behavior [5], [10].

Disinfection makes a major contribution to reducing coliforms and preventing them from being transmitted through water and food [11]. Chlorination is the most commonly used method worldwide and is effective in killing pathogens [11], [12]. However, disinfection by chlorination can produce by-products that are genotoxic to animals and humans [12]. Long-term chlorination needs to be considered, because it causes hemolytic anemia cancer and is toxic to the nervous system and liver [13], [14]. The use of phytochemicals has been widely used and can be an alternative in disinfecting water [15].

One of the plants that has the potential to overcome this problem is Colocasia esculenta L. (C. esculenta) or known as taro. The type of C. esculenta is included in the Araceae family and is known to have properties that contain calcium, phosphorus, thiamine, riboflavin, niacin, oxalic acid, calcium oxalate, sapotoxins, and flavonoids [16]. People in the world have used C. esculenta in traditional medicine, especially in tropical and subtropical areas [17]. Taro is one type of herb in the atlas of Indonesian medicinal plants to treat diseases [18]. Empirically, taro has been used as a medicine; to improve the immune system, reduce cholesterol levels, lose weight, control blood pressure, prevent anemia, rejuvenate the skin, prevent hemorrhage, treat wounds and burns, and overcome infectious diseases [19–21].

Various phytochemical constituents are contained in the tuber and leaf extracts of *C. esculenta* such as tannins, phenols, terpenoids, alkaloids, flavonoids, glycosides, and others [21–23]. However, the phenol group phytosterol is only found in the leaf extract and not found in the tuber extract [22]. This group of phytocomponents has the potential to be an effective antimicrobial, both against gram-positive and gram-negative bacteria [23],[24]. The Colocasia species are known to have significant antidiarrheal activity [25]. *C. esculenta* extract at 25%-30% produces a strong inhibitory effect against *E. coli* [26]. *C. esculenta* is a species that thrives in wetlands and can be efficiently engineered for wastewater treatment [27]. The phytochemical potential of *C. esculenta* may act as an alternative disinfectant. Based on the antibacterial content in *C. esculenta*, the aim of this study was to analyze the ability of *C. esculenta* extract to inhibit Coliform colonization found in river water samples.

2 Materials and methods

This experimental research with a one group pretest-posttest and control design has passed the ethical test with letter number 273/KEPK-FK ULM/EC/IX/2023. The research was conducted at the Microbiology and Parasitology Laboratory, Faculty of Medicine and Health Sciences, Lambung Mangkurat University (FKIK-ULM), in September-December 2024.

The research materials were river water samples, *C. esculenta* leaf extracts at various concentrations, and 0.0002% chlorine (positive control). The media used in the MPN and TPC tests were Lactose Broth (LB), Brilliant Green Lactose Broth (BGLB), and Nutrient Agar (NA). The solvents in the preparation of the extract and test media were 70% ethanol, sterile distilled water, and 0.9% NaCl. The equipment used included test tubes, Bunsen burners, Durham tubes, beakers, scales, droppers, loops, petri dishes, autoclaves, diluent bottles, incubators, autoclaves, and other standard equipment in the microbiology laboratory.

2.1 Phytochemical Screening

The extract of *C. esculenta* leaves underwent qualitative screening to identify phytochemical compounds, following standard protocols with modifications as outlined by Harborne (1987). 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 [28].

2.2 Preparation of Water Samples

The water samples tested were taken from the river in Sungai Lulut, Banjarmasin City, on September 20, 2023 at 07.30 WITA. Aseptic water sampling was carried out at 3 different river flow points, then the water samples were put into sterile bottles, tightly closed, and protected from sunlight. The bacteriological testing procedure for water samples refers to APHA: standard guidelines for water and wastewater examination [29].

2.3 Preparation of Extract *C. esculenta*

Taro plants (*C. esculenta*) were taken from the Pemurus area, Banjarmasin City, in September 2023. Approximately 1.5 kg of fresh, light green taro leaves were prepared and collected to be cleaned, cut into small pieces, and dried in an oven at 50°C. After drying, the leaves were ground into a fine powder and 100 grams of leaf powder was prepared, macerated in 400 mL of 70% ethanol (Merck) while stirring continuously. The extraction process was carried out for three days and an ethanol-free test was carried out using a rotary vacuum evaporator at 50°C. The resulting extract was stored in a labeled airtight container in the refrigerator at 4°C for further use [19]. The extract was made in several concentration groups by adding distilled water (concentrations of 7.5 mg/L, 15 mg/L, 30 mg/L, 60 mg/L, and 90 mg/L) and prepared individually (10 ml) for 3 repetitions.

2.4 Treatment Activity Test based on the Coliform MPN

Suspension of water samples with extracts analyzed based on turbidity in liquid media and growth of bacteria living on agar media. Pour 1000 mL of river water sample into a sterilized beaker. Add 7.5, 15, 30, 60, and 90 (mg/L) of extract, then stir using a jar test at a speed of 120 rpm for 1 minute, continued at a speed of 30 rpm for 20 minutes, then left for 30 minutes. Filter the supernatant, then analyze the total coliform using the MPN (Most Probable Number) method with SNI-01-2332.1-2006. The testing stages include presumptive tests, confirmation tests, and total plate numbers (TPC).

Presumptive Test: Add 10 mL of sterile pipette into an LB Series 5 tube with a Durham tube and incubate for 48 hours at 35°C-37°C. If the tube shows turbidity and gas in the Durham tube, proceed with a confirmation test. If negative, incubate for an additional 24 hours. Confirmed Test: Inoculate positive LB tubes into BGLB tubes containing a Durham tube using a loop. Incubate the inoculated BGLB tubes for 48 hours at 35°C-37°C. Examine the BGLB tubes at 35°C-37°C for gas production; positive tubes will show both turbidity and gas in the Durham tube. Determine the MPN value based on the number of positive BGLB tubes using the MPN table. The results are reported in CFU/100 mL of the sample [30]. The TPC in water samples using the dilution method. In a tube containing a water sample with the addition of extract, 10 ml of suspension was pipetted and put into a cup, and warm NA (40 ℃) was added. Then incubate all cups for 2 x 24 hours (37 ℃) [31]. Count the number of growing colonies.

2.5 Statistical Analysis

The research data were analyzed and obtained in normal and homogeneous distribution. Analysis of parameteric data was carried out using ANOVA and Duncan's post-hoc test with a 95% confidence level [32].

3 Results and discussion

This study assessed the bacteriological quality of river water samples before and after taro extract administration, based on the MPN Coliform test (presumptive and confirmed tests) and the TPC test. An example of the observation results is shown in Figure 1.

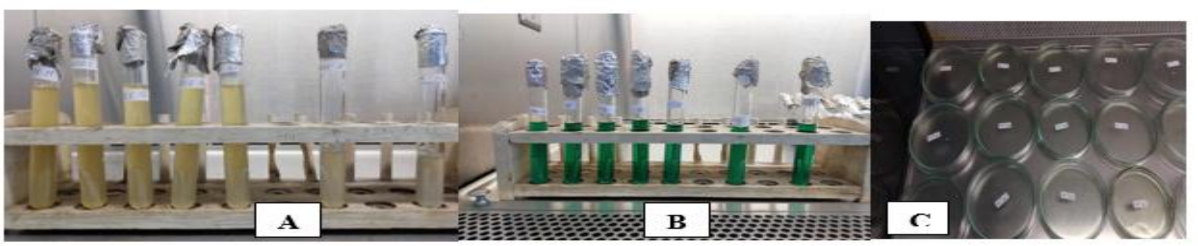
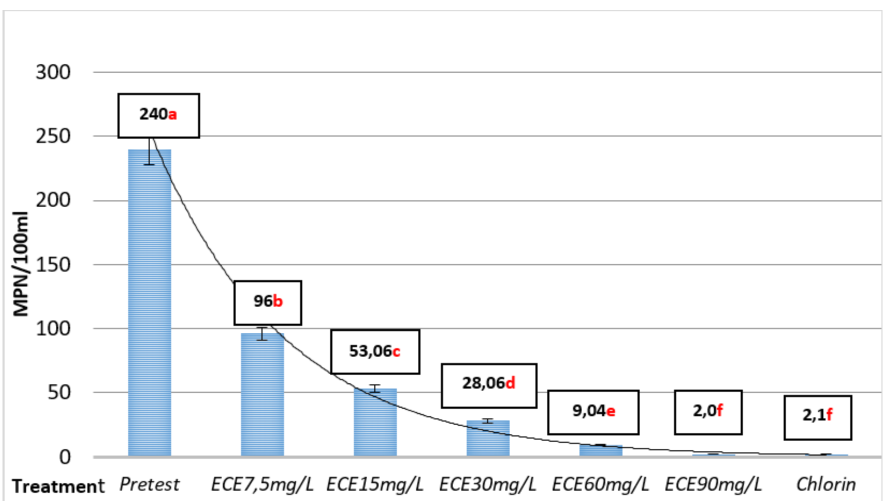


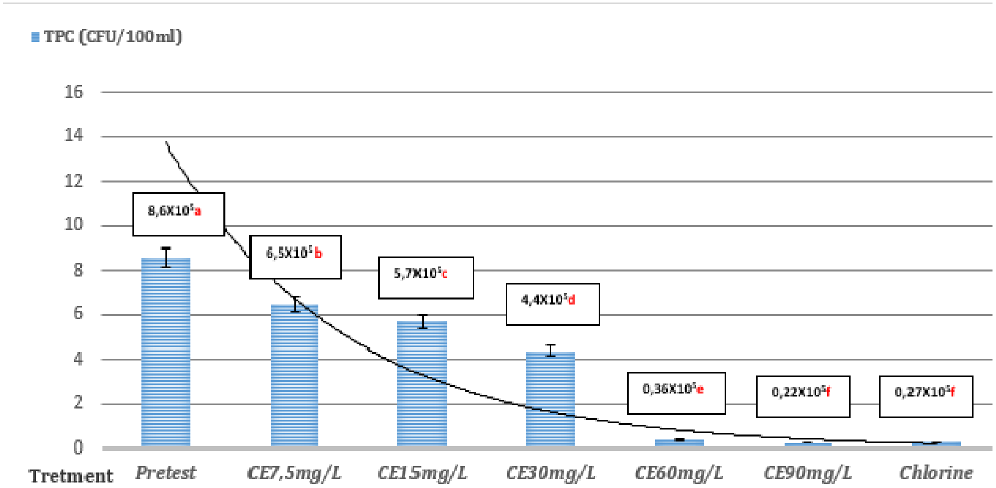
Fig. 1. Results of Coliform Observations of Water Samples in MPN and TPC Tests: A. Presumptive Test; B. Confirmed Test; C. TPC Test

The results of MPN observations and the average TPC values of water samples before and after the administration of extract and control are shown in Figure 2 and Figure 3. There is a difference in the Coliform value of water samples between before and after the administration of taro extract. The results of observations on the Lulut River water sample before the extract treatment, were found to contain coliform bacteria with an MPN of 240 MPN/100ml and a total of $86x10^5$ CFU/100ml of sample. This value indicates that the total coliform content in the river water sample has exceeded the quality standard. According to SNI 01-3553-1996, the requirement for drinking water circulating on the market is to have a maximum total plate count (TPC) of 1.0 $CFUx10^5$. According to Permenkes No. 736 of 2010, the number of bacteria allowed in piped water samples is 10 CFU/100 ml, while in non-piped water samples it is 50 CFU/100 ml [33]. Coliform is an enter-pathogen or can cause diseases in the digestive tract such as diarrhea, so water disinfection is needed.



Description: ECE = C. esculenta extract. Different letters in the MPN value indicate a different effect based on the Duncan test at a significant level of 5%.

Fig. 2. The average MPN Coliform values (CFU/100ml) of test water samples before and after treatment



Description: ***ECE = C. esculenta extract.*** **Different letters in the TPC value indicate a different effect based on the Duncan test at a significant level of 5%.**

Fig. 3. The average TPC (CFU/100ml) of test water samples before and after treatment

Fig. 2 and 3 show the effect of *C. esculenta* extract in inhibiting coliform growth significantly. Referring to health requirements regarding air and the results of statistical analysis, it shows that the effectiveness of *C. esculenta* extract 7.5mg/L – 30mg/L has not produced an effect according to health standards. The 60mg/L extract began to provide a reduction effect on coliforms in water samples and the 90mg/L extract produced an effectiveness equivalent to chlorine. Increasing the concentration of the extract significantly reduced coliform colonization.

The activity of *C. esculenta* in inhibiting the growth of coliform bacteria is due to the role of antibacterial compounds. [33]. The group of bioactive compounds identified in taro extract in this study is the same as that reported by Elmosallamy et al., namely compounds of the phenol, alkaloid, flavonoid, tannin, saponin, steroid, and terpenoid groups [34]. These results are relatively the same as those found in the extract gel preparation [35]. However, the bioactive content can differ in extracts with methanol, aquades, and 96% ethanol solvents [19], [20]. In addition, variations in phytochemical content/composition can be caused by differences in the geographical origin of the plant which affect phytoconstituents [36].

Many biological properties of phytochemicals have been reported, including antimicrobial activity [34]. The main mechanism of action of flavonoids against *E. coli* is inhibition of nucleic acid synthesis (DNA gyrase) and damage to cell membranes which will cause changes in membrane fluidity followed by bacterial cell lysis [37]. Alkaloids can damage the integrity of the outer membrane and cytoplasmic membrane of bacteria, by penetrating the reconstituted LPS monolayer, depolarizing the cytoplasmic membrane, causing leakage of cytoplasmic contents, as well as an outward pump that is negative for porin [38]. The activity of phenolic compounds on gram-negative bacteria causes a downregulation of the expression of genes regulated by the type III secretion system; these genes are related to the invasion capacity, intracellular survival and bacterial multiplication [39]. Tannins interact with substrate proteins and the plasma membrane of bacterial cell walls, by binding metal ions, thus inhibiting bacterial cell growth and metabolism [40]. Saponins have the effect of affecting the integrity of bacterial cell membranes and causing irreversible loss of intracellular components [41]. Steroid and terpenoid compounds have the effect of destroying cell membranes (lipophilic properties), inhibiting ATP and enzymes in cell membranes, and inhibiting protein synthesis pathways [42].

Disinfectants produced from plant extracts have several advantages over conventional chemicals [27]. The results of this study indicate that *C. esculenta* extract has antimicrobial properties in river water samples; however, the practicality of the extract for drinking water disinfection needs to be studied further. This is because drinking water disinfectants that meet national health standards and the World Health Organization set zero *E. coli* or thermotolerant coliforms in 100 mL of water samples after treatment [31], [33]. The use of chlorine as a disinfectant also needs to be evaluated because of the development of biofilm-forming *E. coli* strains that are more resistant to antimicrobials [43]. To increase the activity of organic matter hydrostatically to the cell membrane as an antimicrobial, it can be combined during the water disinfection filtration process [44].

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Taro leaf extract does not contain anthraquinone and is classified as non-toxic, and is permitted for use up to a level of 5000 mg/kg. The advantage of using natural disinfectants is that they are more environmentally friendly and biodegradable, and without harmful residues [36], [45], [47]. The presence of *C. esculenta* which thrives in wetlands; makes taro leaves potentially developed as an alternative disinfectant [27]. This study is limited to in vitro studies; the effectiveness and role of taro leaf extract as a disinfectant for disease prevention and control, requires further research.

4 Conclusion

Colocasia esculenta leaf extract contains several bioactive compounds that act as antibacterials to inhibit the colonization of Coliform bacteria. Administration of C. esculenta extract 60-90mg/L in river water samples has been proven in vitro to be able to reduce MPN and TPC Coliform values and the effectiveness of chlorine control. The results of this study make the potential of C. esculenta leaf extract to be developed as an alternative disinfectant in reducing the colonization of coliform bacteria in river water. Further testing stages are needed as a disinfectant that is supported by the safety of its use.

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