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Research Article

Inhibition Of Leaf Extract of Rubber Plant Against Enterobacteriaceae Isolated from Drinking Water Refill

Sri A. F. Kusuma[1*], Novia B. Kartika[1], Danni Ramdhani[2]

[1]Departemen Biologi Farmasi, Fakultas Farmasi, Universitas Padjadjaran, Sumedang, West Java, Indonesia 45363,

[2]Departemen Analisis Farmasi dan Kimia Medisinal, Fakultas Farmasi, Universitas Padjadjaran, Sumedang, West Java, Indonesia 45363

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*Corresponding author: s.a.f.kusuma@unpad.ac.id

Abstract

This study was aimed to identify enterobacteria strains in drinking water refill from 20 refill water stations in Jatinangor, West Java, and investigated the potential antibacterial activity of rubber plant (*Ficus elastica* Roxb. ex Hornem) leaf extracts on identified strains of the isolated enterobacteria. Bacterial isolation was accomplished using membrane filtration method and the bacterial identification was performed by observing the cell and colonies morphologies, biochemical testing approach and concluded using the computer program Global Infectious Diseases and Epidemiology Network (GIDEON). The antimicrobial susceptibility test was carried out using the Kirby-Bauer diffusion technique. From 20 water samples, 30 single colonies were isolated and identified, as follows: *Citrobacter freundii* (nine samples), *Salmonella typhi* (six samples), *Serratia marescens* (six samples) and *Escherichia coli* (nine samples) with percentage: 30%, 20%, 20% and 30%. The extract can inhibit all Enterobacteria isolates, however, among those strains, *E. coli* was the most susceptible Enterobacteria to be inhibited by *F. elastica* leaf extract. The current study suggests that *F. elastica* leaf extract potentially to be used as a novel antibacterial agent against Enterobacteria strains as the major gastrointestinal tract pathogenic microbes.

Keywords: Antibacterial, drinking water refill, Enterobacteriaceae, *Ficus elastica* Roxb. ex Hornem, isolation.

Penghambatan Ekstrak Daun Karet Kebo Terhadap Isolat Enterobacteriaceae dari Air Minum Isi Ulang

Abstrak

Penelitian ini bertujuan untuk mengidentifikasi spesies enterobacteria dalam air minum isi ulang dari 20 depot air isi ulang di Jatinangor, Jawa Barat, dan menyelidiki potensi aktivitas antibakteri ekstrak daun tanaman karet kebo (Ficus elastica Roxb. ex Hornem) terhadap isolat enterobacteria tersebut. Isolasi bakteri dilakukan dengan metode filtrasi membran dan identifikasi bakteri dilakukan dengan mengamati morfologi sel dan koloni, pendekatan pengujian biokimia dan disimpulkan dengan menggunakan pendekatan program komputer *Global Infectious Diseases and Epidemiology Network* (GIDEON). Uji kepekaan antibiotik dilakukan dengan teknik difusi Kirby-Bauer. Dari 20 sampel air isi ulang, sebanyak 30 koloni tunggal telah diisolasi dan teridentifikasi, sebagai berikut: *Citrobacter freundii* (sembilan sampel), *Salmonella typhi* (enam sampel), *Serratia marescens* (enam sampel) dan *Escherichia coli* (sembilan sampel) dengan persentase: 30%, 20%, 20% dan 30% dari jumlah koloni total. Berdasarkan uji aktivitas antibakteri, ekstrak daun *F. elastica* mampu menghambat seluruh isolat Enterobacteria, namun diantara strain tersebut, *E. coli* merupakan enterobacteria yang paling rentan dihambat oleh ekstrak daun *F. elastica*. Dengan demikian, dapat disimpulkan bahwa tanaman ini berpotensi sebagai agen antibakteri baru terhadap spesies Enterobacteria sebagai mikroba patogen utama saluran pencernaan.

Kata Kunci: antibakteri**,** air minum isi ulang, Enterobacteriaceae, *Ficus elastica* Roxb. ex Hornem, isolasi.

1. Introduction

If water is pathogen-free, it is deemed safe to drink. The requirement for risk-informed planning to ensure the safety of drinking water is very important. It is advised that drinking water be tested on a regular basis to verify its safety. Drinking water is obtained from a variety of sources in urban houses.[1] Drinking water is water that has gone through a processing process or without a processing process that meets health requirements and can be drunk directly. Drinking water is safe for human consumption if it fulfills the physical, microbiological, chemical, and radioactive conditions specified in the mandatory and supplementary parameters.[2] A pipe network system provides drinking water; non-pipeline networks provide drinking water from shallow wells, hand pump wells, rainwater storage tanks, water terminals, water tank vehicles, or building/spring protection; bottled drinking water and refill drinking water. Every effort should be made to attain the highest possible level of water safety.[3]

The most serious health concern connected with water-borne microbial infections is diarrhea. Pathogenic Enterobacteriaceae members causing gastroenteritis have been identified from both recreational and drinking waters.[3] The presence of Enterobacteriaceae members in water has been regarded a sign of faecal pollution.[4] Resistant bacteria can pollute human drinking water supplies. Ampicillin resistance was found in 98-100% of Enterobacteriaceae isolated from drinking water. Multiple antibiotic-resistant *E. coli* (35%), Salmonella (22.7%), and Shigella (15%) were discovered in the drinking water.[5] Antibacterial resistance in pathogenic bacteria and contaminated water are contributing to the spreading of infectious diseases.[1] As a result, isolation and antimicrobial susceptibility testing for drinking water samples are becoming increasingly important in addressing the human health hazards associated with ingesting contaminated water. Because of the emergence of multidrug resistance in Enterobacteriaceae, the rapid emergence of new infections, and the potential for use of multidrug-resistant agents in bioweapons.[6] The need for new antimicrobial agents and strategies for their use in the treatment of serious Enterobacteriaceae infections is evident.[7-9] Therefore, we isolated and identified the Enterobacteriace from drinking water refill obtained from 20 refill water stations in Jatinangor, West Java, Indonesia as the information data of major contaminants to and investigated the potential antibacterial activity of rubber plant (*Ficus elastica* Roxb. ex Hornem) leaf extracts on identified strains of the isolated enterobacteria. The identity of the type of bacterial species contaminating the refill water can be used as a database to facilitate the strategies to decontaminate dringking water refill. In this study, standardized *F. elastica* extracts which have been utilized in traditional medicine to treat wounds and other topical infections, was explored as the natural antibacterial candidate to inhibit those isolated Enterobacteriaceae due to its reported antibacterial phytochemical contents that include tannins, flavonoids, and phenols that have an antibacterial mode of action.[10,11]

2. Methods

2.1. Materials

The plant materials used in this study were the leaves of *Ficus elastica* Roxb. ex Hornem from Jatinangor, West Java and it had been authenticated (no. 165/HB/03) at Determination institution, Department of Biology, FMIPA, Padjadjaran University, Indonesia. The source of Enterobacteriaceae strains isolates was refill drinking water taken from 20 different sources in the Jatinangor area, West Java, Indonesia. The growth media used in this research were Macconkey Agar (Oxoid), Mueller-Hinton Agar (Oxoid), Eosin Methylene Blue Agar (Oxoid), Salmonella Shigella Agar (Oxoid), Brilliant Green Agar (Oxoid), X.L.D. Agar (Oxoid), Kligler Iron Agar (Oxoid) and Mueller Hinton Broth (Oxoid).

2.2. Prosedure

2.3.1. Extract Preparation

Fresh leaves of F. elastica was finely

chopped to create smaller simplicia pieces, then dried in an oven at 60°C for 24 h, crushed to a powder, and ready for extraction.[12] 1.5 kg of leaf powder was weighed and macerated in 70% ethanol for 3x24 h in a macerator, with the solvent replenished every 24 h and alternated with stirring.13 The liquid extract was collected and mixed every day to obtain the complete extract. All liquid extracts were concentrated using a rotary evaporator at 40-50°C, evaporated under vacuum pressure at 60°C and floated over a water bath until a constant mass was produced. The acquired yield percentage was then calculated.

2.3.2. Extract Standardization

Standardization of the ethanol extract of *F. elastica* leaves was carried out by determining several specific and non-specific parameters. The specific parameters performed included organoleptic extract (shape, color, and taste), phytochemical screening and thin layer chromatography (TLC). A phytochemical screening was carried out to assess the secondary metabolite compounds in *F. elastica* leaf ethanol extract. The phytochemical screening included alkaloids, tannins and polyphenolics, flavonoids, monoterpenoids, sesquiterpenoids, steroids and triterpenoids, quinones, and saponins. Meanwhile for TLC, it was carried out to identify the active compounds contained in the ethanol extract of *F. elastica* leaves. TLC was carried out using a thin layer plate with a stationary phase in the form of silica gel 60 F254 and the mobile phase was toluene: ethyl acetate (7:3v/v). The development results are detected with UV light 254 and 366 nm and will obtain an Rf value visible from the detected spots. Non-specific parameters carried out include determining water content, total ash content, and acid insoluble total ash content. Those tests were performed using standard procedure.[14]

2.3.3. Isolation and Identification of Enterobacteriaceae Strains

Enterobacteriaceae colonies were isolated using Mac Conkey Agar medium then the isolation process was done repeatedly until a pure isolate was obtained and ready to be identified. Bacterial identification was accomplished in phases, beginning with evaluating the morphology of bacterial colonies, followed by Gram staining and physiological testing using a serial of biochemical assays. Then, the identities of the isolates were concluded using the computer program Global Infectious Diseases and Epidemiology Network (GIDEON).

2.3.4. Antibacterial Activity Test

The agar diffusion technique was used to test the antibacterial activity of the extract. The leaf extract stock solution was serially diluted starting at 80% to 40% w/v in 10% DMSO. Bacterial suspension was made by inoculating 2 or 3 Ose of bacterial slant agar colonies into 5 ml of sterile physiological NaCl. The bacterial suspension's turbidity was adjusted to produce a concentration of 1.5 x10^8 CFU/mL (0.5 McFarland's standard). A 20 µL produced bacterial suspension was put into a sterile petri-disc containing 20 mL liquid MHA, homogenized, and solidified. The inoculation plates were then perforated to provide holes for extract storage in a volume of 50 µL at a certain concentration (20-80%w/v). After that, the plates were incubated at 37°C for 24 h. The diameter of inhibition zones was measured.

3. Result

3.1. Extraction Results

The quantity of extract produced minus the number of original samples obtained prior to extraction is known as extract yield, and it is stated as a percentage.15 The extraction method must be finished before analyzing the yield of the *F. elastica* extract. *F. elastica* was extracted using a single maceration method. The obtained concentrated extract was 18.58% using 70% ethanol as a solvent on old leaves utilizing a sample of 1500 g of dry weight of old *F. elastica* leaf. The amount of yield is determined by the solubility qualities of the bioactive components.

3.2. Standardization: Specific Parameter

The organoleptic test uses the sense

to reveal features quickly, easily, and objectively. The form, odor, color, and taste of *Ficus elastica* leaves extract were described as organoleptic criteria. The separation procedure produced a thick extract, and the solvent had totally evaporated. The extract's secondary metabolite components generated the bitter flavor. According to the phytochemical findings, the ethanolic extract includes steroid, flavonoid, polyphenolics, saponins, quinones, and tannins. The TLC profile determined from the ethanol extract of *F. elastica* leaves may be observed in the Table 1 and Figure 1.

3.3. Standardization: Non-specific Parameter

The non-specific parameter includes water content (5±0.00% v/b), total ash content (12.5±0.01 % b/b), acid insoluble ash content (1.25±0.02 % b/b), and loss on drying (20±0.00 (% b/b).

3.4. Isolation and identification results of Enterobacteriaceae strains

To understand the morphological and biochemical characteristic of the contaminated refilled drinking water, we performed morphological tests, and TSIA tests on the samples. From 20 drinking-water refill stations, 30 single colonies were found and the major species was categorized as 4 species. The colonies morphology was observed using specific mediums, including Mac Conkey Agar, Eosin Methylene Blue Agar, and Salmonella Shigella Agar, shown in Table 2. All single colonies displayed the same shape (round), texture (convex, except colony no.3) and edge shape (regular), but shown distinct color.

The morphological characteristics of all isolated strains were rod-shaped, in red color, Gram-negative, and non-spore-forming bacteria. The IMViC tests results were variative between those four strains, presented in Table 3. The indole test showed that all of the samples were negative, which was shown by the absence of a red ring on the media layer. While the motility test, MR, VP, and citrate tests exhibited various results. The TSIA test showed that there were red (positive) and yellow (negative) slant production, while all samples show yellow (negative) butt production. The gas production and the H_2S assessment was showing various results. Based on morphological colony and biochemical test, 4 isolated spesies, consist of 30% (n=9) were positive for *Citrobacter sp*; 20% (n=6) were positive for *Salmonella sp*; 20% (n=6) were positive for *Serratia sp* and 30% (n=9) were positive for *Escherichia sp*.

According to the results of the identification test performed on the four bacterial isolates using the GIDEON software, can be seen in Table 4. The identity of bacterial isolate 1 is *Citrobacter freundii* with a similarity percentage of 91%, bacterial isolate 2 is *Salmonella enterica* serovar. typhii with a similarity percentage of 99%, and bacterial isolate 3 is *Serratia marcescens* with a similarity percentage of 94%.and isolate 4 is *Escherichia coli* with 93% as the similarity percentage.

3.5. Antibacterial Activity Results

The inhibitory effect of the *F. elastica* leaf extract on some strains of enterobacteria is indicated in Table 5. There was significant difference on the inhibitory effect of the different concentrations of F. elastica on the enterobacteria strains investigated, even though physicochemical characteristics

Table 1. TLC of *F. elastica* leaf extract

Rf	Spot color on UV wavelength	
	254 nm	366 nm
0.57	-	Greenish yellow

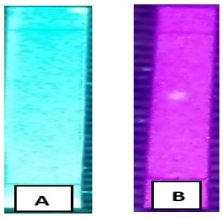

Figure 1. TLC of *F. elastica* leaf extract on certain UV wavelength
Notes: A. 254 nm and B. 366 nm

Table 2. Color of isolated single colony

Colony	Morphology		
	Mac	Eosin	Salmonella
1	pink	blackish purple	pink
2	colorless	colorless	colorless
3	colorless	colorless	colorless
4	pink	metallic green	pink

varied among samples.

4. Discussion

Contaminated of drinking water poses an enteric disease risk, and the abundance of thermotolerant bacteria identified in this study highlights the need for research into waterborne illness and strategies to enhance water quality in the study community. Water quality interventions have been linked to a reduction in diarrheal illness.[16,17] Previous research has revealed a link between bacterial contamination of drinking water and diarrheal illness incidence. However, studies utilizing coliform levels or *E. coli* to measure water quality show significant heterogeneity.[17] The link between thermotolerant coliforms or even particular indicator bacteria and sickness is complicated, with disease outcomes of interest produced by several pathogens via different exposure pathways.[18] The presence of coliform bacteria in drinking water often indicates that the water was polluted with either human or animal feces. Coliform bacteria are enteropathogenic bacteria that harm human health.[3] The study's samples revealed the formation of coliform bacteria colonies. The Health Minister's Regulation established a maximum limit of coliform bacteria of 0 bacteria per 100 mL of water. This means that the presence of coliform bacteria in drinking water implies that the water is contaminated.[19] Coliforms are an important group of the family Enterobacteriaceae, which constitute about 10% of intestinal microflora.[20] Microbial contamination is possible throughout the water-refilling process, with operator hygiene and cleanliness of processing equipment serving as contamination causes. The discrepancies in the prevalence of coliform groups in water samples might be attributed to changes in sample size and water source between investigations. This information data is very important to be handled due to the role

Table 3. Biochemical assay results

Test	Isolates			
	1	2	3	4
Voges Proskauer	-	-	-	-
Methyl-Red	+	+	+	-
Indole	-	-	-	-
Motile	-	+	-	-
Saccharose	+	+	-	+
Mannose	+	+	+	+
Maltose	+	+	-	+
Lactose	+	-	-	+
Glucose	+(gas)	+	+	+(gas)
Simmons citrate	+	-	+	-
Triple sugar iron agar (TSIA)	+	+	-	+
Urea	-	-	-	+
H_2S	+	+	-	+
TSIA Gas	+	+	-	-

Notes: (+) positive result; (-) negative result

Table 4. GIDEON interpretation data

	Identity	Similarity Probability (%)
Isolate 1	*Citrobacter freundii*	91
	Citrobacter braakii	5
	Citrobacter youngae	3
	Citrobacter gillenii	2
Isolate 2	*Salmonella enterica* serovar. typhii	99
	Citrobacter youngae	<1
	Salmonella enterica subsp. enterica	<1
	Citrobacter braakii	<1
Isolate 3	*Serratia marcescens*	94
	Pantoea agglomerans	2
	Klebsiella pneumoniae	2
	Providencia stuartii	1
Isolate 4	*Escherichia coli*	93
	Citrobacter freundii	5
	Citrobacter farmeri	1
	Aeromonas hydrophila	<1

of those Enterobaceriaceae bacteria species which implicated in gastrointestinal diseases. The incidence of gastrointestinal illnesses has increased significantly in both emerging and developed countries across the world.[21] When enteric bacterial species infiltrate and colonize the digestive system, they can cause gastrointestinal illnesses ranging from diarrhoea, gastroenteritis, shigellosis, and salmonellosis to life-threatening outcomes. Completed test should be conducted to ensure the results of the confirmation test by detecting the nature of fermentation and observing the coliform characteristics. The morphological characteristic and the IMViC methods results were used to differentiate *E. coli* and other Enterobacteriaceae.[22] We also used the tubed differential medium triple sugar iron (TSI) agar is used to determine carbohydrate fermentation and H_2S generation. Carbohydrate metabolism gas can also be observed. Bacteria can either aerobically (with oxygen) or fementatively (without oxygen) digest carbohydrates. TSI classifies bacteria according on their ability to ferment lactose, glucose, and sucrose as well as produce hydrogen sulfide. Although it is beneficial for other Gram-negative bacteria, TSI is most commonly employed in the detection of Enterobacteriaceae.[23] This study found that the bacteria identified were Citrobacter freundii, *Salmonella enterica* serovar. Typhi (S. typhi), S*erratia marcescens* and *Escherichia coli*, which are included in the members of the Enterobacteriaceae family. Our finding of Enterobacteriaceae strains showed different isolates identity of the contaminants from other study. Bacteriological examinations on drinking-water samples collected from refill stations in Jagakarsa, Jakarta, revealed the presence of S*taphylococcus aureus*, but no *E. coli*, *Salmonella*, *Clostridium perfringens*, or Pseudomonas aeruginosa were found.[24] Different species such as Proteus sp, *E. coli* and *Klebsiella oxytoca*, were reported from drinking water refill stations of Kendari, Indonesia.[25] By comparing those data, we may conclude that unsanitary environmental conditions are the primary source of bacterial infection. The identified bacteria from our study are pathogenic bacteria in Enterobacteriaceae family. *Citrobacter freundii* is an inhibitant of both human and animal digestive systems.[26] This bacterium frequently triggers serious opportunistic infections and have been reported as the causative agent of newborn meningitis and brain abscesses.[27] *C. freundii* infections

Table 5. Antibacterial activity results

Extract concentration (%) (b/v)	Diameter of inhibition (mm) against:			
	C. freundii	*S. enterica*	*S. marcescens*	*E. coli*
80	11.36±0.02	11.69±0.01	11.57±0.03	15.69±0.14
60	9.80±0.01	10.24±0.04	10.35±0.15	12.02±0.02
40	8.13±0.12	8.91±0.03	9.69±0.03	10.57±0.01

related to the urinary tract, meningitis, sepsis, pneumonia, diarrhea, respiratory and wound infections.[28] *C. freundii* isolates also reported to be resistant to ampicillin, cefazolin, cephalothin, cefoxitin, aboren, doxycycline, neomycin, penicillin, erythromycin, and vancomycin.[29,30] Therefore, *C. freundii* has emerged as a pathogen of concern nowadays. Next identified bacteria, *S. typhi* is considered the main cause of typhoid fever, which is generally transmitted by the consumption of contaminated food and/or water.[31] Currently, the emergence of antimicrobial-resistant *S. typhi* strains presents an enormous obstacle to its successful treatment.[32] The emergence of multidrug-resistant (MDR) *S. typhi*, resistant to standard first-line antibiotics such as chloramphenicol, ampicillin, and trimethoprim/sulfamethoxazole, has led to the adoption of fluoroquinolone antibiotics such as ciprofloxacin as first-line therapy. However, due to fluoroquinolone resistance, notably in South Asia, third-generation cephalosporins (e.g., ceftriaxone) are now utilized as first-line therapy.[33] Whereas the importance of the *S. marescens* presence in contaminated drinking water can potentially cause several infection diseases such as pneumonia, sepsis, meningitis, peritonitis, endocarditis, arthritis, osteomyelitis, keratitis, and urinary tract and skin infections.[34-37] Because of its inherent resistance to antibiotics such as ampicillin, first and second generation cephalosporins, macrolides, and cationic antimicrobial peptides (CAPs), clinical management of *S. marcescens* infections is difficult.[38,39] As a result, patients in the intensive care unit with those antibiotics regimen are at a higher risk of contracting infections caused by this bacterium. The last identified and commons found in contaminated water, *E. coli*. *E. coli* is a bacterium with a unique place in the microbiological world since it not only causes serious illnesses in people and animals, but it also contributes significantly to the autochthonous microbiota of the various hosts.[40] Aside from β-lactam resistance, *E. coli* resistance includes sulphonamides, trimethoprim, and ciprofloxacin.[41] Antibacterial resistance in pathogenic bacteria and contaminated water are contributing to the spreading of infectious diseases.[1] As a result, drinking water sample separation and antimicrobial susceptibility testing are becoming increasingly crucial in addressing the human health risks connected with eating polluted water. Because of the emergence of multidrug resistance in Enterobacteriaceae, the rapid emergence of new infections, and the potential for multidrug-resistant agents to be used in bioweapons. There is an urgent need for new antimicrobial agents and strategies for their use in the treatment of serious Enterobacteriaceae infections due to the contaminated drinking water consumption. Therefore, we investigated the potential antibacterial activity of *F. elastica* leaf extracts on identified strains of the isolated Enterobacteria. Standardized *F. elastica* extracts have been utilized in traditional medicine to treat wounds and other topical infections.[10]

Standardization is a procedure that ensures that every drug, particularly herbal medicines, that has been sold has active ingredients at the appropriate quantity or dose and will generate a therapeutic effect.[42] This is a critical stage in maintaining the consistency of biological activity, chemical profile, or simply a quality assurance of medicinal ingredient for manufacture and herbal medicine product.[43] Additionally, extract standardization can boost the commercial worth of herbal medication

producers.[44] Standardized extracts are high-quality extracts that have been utilized as raw materials in the manufacturing of herbal medicines and have passed quality control tests from planting through harvesting to the manufacture of herbal products. Based on the result of standardization of specific and non-specific parameters, the ethanolic extract of *F. elastica* leaves has met the specified requirements. According to the Indonesian Herbal Pharmacopoeia, the allowed water content is less than 10%. In this investigation, the water content of the ethanolic 70% extract of *F. elastica* leaves was 5 %, therefore meeting the requirements. The goal of calculating the water content in the extract is to set a minimum limit for the quantity of water in the substance (extract). The higher water content of the extract encourages the growth of fungus and molds, which might limit the activity of the active component in the herbal extract. Total ash was also evaluated to provide an overview of internal and external mineral content from the beginning to obtain the extract. Total ash content of the ethanolic 70% extract of *F. elastica* was 12.5±0.01 %b/b. According to Herbal Pharmacopoeia Indonesia, the requirement of extract total ash content maximum at value of 13.3%b/b.45 From phytochemical analysis, we found steroid, flavonoid, polyphenolics, saponins, quinones, and tannins in the *F. elastica* leaves ethanolic extract. Those substance have been reported their efficacy as antimicrobial and resistance modifiers.[45] Those phytochemicals may suppress microbial growth by disrupting cellular membranes, interfering with specific microbial metabolic activities, or modulating signal transduction or gene expression pathways.[46] The antibacterial mechanism of steroid is blocking bacterial cell membrane function and producing a complex extracellular protein molecule. In addition, steroid can cause the release of the intracellular substance by disrupting the cell membrane.[47] Flavonoids inhibit microbial enzymes, energy metabolism, cytoplasmic membrane function, porins in cell membranes, nucleic acid synthesis, inhibit biofilm attachment and formation, and altering the membrane permeability.[48-49] Meanwhile, the antibacterials mechanisms of phenolic are denaturing cell proteins and blocking bacterial nucleic acid production.[50] The action of saponins by limiting bacterial growth by reducing surface tension, which increases cell permeability and allows intracellular chemicals to be released.[51] Tannins suppress bacterial development by acting as a siderophore, chelating iron from the substrate and rendering it inaccessible to bacteria.[52] Quinones have important roles as redox mediators in a variety of electron-transfer processes in living organisms.[53] Therefore, according to Table 5, it clearly showed *F. elastica* extract potentially inhibit all Enterobacteria isolates. Davis and Stout grouped the criteria for antibacterial power as follows: an inhibition zone diameter of 5 mm or less was categorized as weak, an inhibition zone of 5-10 mm was classified as moderate, an inhibition zone of 10-20 mm was categorized as strong and an inhibition zone of 20 mm or more was categorized as very strong antibacterial. The extract showed moderate to strong inhibition against all bacterial isolates and among those strains, *E. coli* was the most susceptible to be inhibited by the extract, indicated by the highest diameter of inhibition value.

5. Conclusion

Based on the characteristics observed from the microbiology and biochemistry testing, it was concluded that among 20 drinking-water refill stations in Jatinangor, all were contaminated by at least 1 species of Enterobacteriaceae bacteria. This study found that the bacteria identified were *Citrobacter freundii*, *Salmonella enterica* serovar. typhi, Serratia marcescens and *Escherichia coli*. Continuous supervision to the refilled drinking-water quality is needed to ensure the quality of drinking-water in Jatinangor and the ethanol extract of *F. elastica* leaves can be effective as an antibiotic against illnesses caused by those identified Enterobacteriaceae strains.

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