BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 13, Number 2, October 2024 | Pages: 449-458 | DOI: 10.14421/biomedich.2024.132.449-458 ISSN 2540-9328 (online)

Antibacterial Activity of Sweet Orange (*Citrus sinensis*) Peel Tea against *Enterobacteriaceae* Isolated from a Water Depot

Yumiko[1], Suhartomi[2], Sri Wahyuni Nasution[3,*], Siti Syarifah[4], Ade Pryta R. Simaremare[5]
[1]Medical Student Programme, Faculty of Medicine, Dentistry, and Health Science, Universitas Prima Indonesia, Medan, Sumatera Utara, Indonesia.
[2]Departement of Pharmacology, Faculty of Medicine, Dentistry, and Health Science, Universitas Prima Indonesia, Medan, Sumatera Utara, Indonesia.
[3]Departement of Tropical Medicine, Faculty of Medicine, Dentistry, and Health Science, Universitas Prima Indonesia, Medan, Sumatera Utara, Indonesia.
[4]Departement of Pharmacology and Therapeutic, Faculty of Medicine, Universitas Sumatera Utara, Medan, Sumatera Utara, Indonesia.
[5]Departement of Microbiology, Faculty of Medicine, Universitas HKBP Nommensen, Medan, Sumatera Utara, Indonesia.

Corresponding author*
sriwahyuninasution@unprimdn.ac.id

Manuscript received: 22 Agustus, 2024. Revision accepted: 10 October, 2024. Published: 17 October, 2024.

Abstract

Most orange peels are not utilized and become useless waste that is thrown away. Orange peels contain many nutrients and compounds, such as flavonoids, terpenoids, alkaloids, and essential oils, which are secondary metabolites that act as antibacterial agents. This study intends to investigate the antibacterial activity of sweet orange peel tea against isolated *Enterobacteriaceae* from a random water depot sample around Universitas Prima Indonesia. This experiment used disc diffusion for antibacterial assay. Meanwhile, the sweet orange peel was brewed using two different methods, including infusion and decoction, in two different masses (3 grams and 5 grams). These sweet orange peel tea formulations were compared to standard (chloramphenicol) and control (distilled water). Enterobacteriaceae was isolated and identified from a random water depot sample around Universitas Prima Indonesia, which included colony identification in EMB agar and MacConkey agar, gram staining, and biochemical test. This study showed that a random water depot sample contaminated by *Enterobacteriaceae* had properties similar to *Citrobacter sp*. Sweet orange tea formulation inhibited this isolated *Enterobacteriaceae* growth (P-Value: 0.010). Antibacterial activity was observed in 3-gram infusion, 5-gram infusion, and 5-gram decoction. However, the antibacterial activity was not better than the standard (chloramphenicol). Overall, it can be concluded that the sweet orange peel tea as infusion or decoction has weak antibacterial activity against Enterobacteriaceae bacteria isolated from water depots, which had some properties similar to *Citrobacter sp.*

Keywords: Sweet Orange; Decoction; *Enterobacteriaceae*; Citrobacter; Infusion.

INTRODUCTION

Infectious disease is a common health burden in some developing countries, primarily caused by bacteria. The agents can cause infectious diseases, including bacteria, viruses, fungi, and parasites. World Health Organization (WHO) reported in 2006 an increase in mortality in some ASEAN countries, which increased to 45%, and in the same year, WHO also reported that the mortality rate of infectious disease was 25 million worldwide. Indonesia, as part of the ASEAN country, is the second-rank country with the most infectious diseases and has a mortality rate of 29.5%. Both agent factors and environment and host play an important role in infectious disease, significantly when human immunity weakens (Damanik et al., 2021).

Enterobacteriaceae is a negative-gram bacteria with a rod shape. The bacteria group of *Enterobacteriaceae* acts as resident flora in the human and animal gastrointestinal (Riedel et al., 2019). *Enterobacteriaceae* are one of the groups of bacteria that can cause diarrhea and often contaminate food (Parawidnyaningsih et al., 2023) and drinks that have been cooked, frozen or not cooked and frozen (Diah et al., 2022).

There are several types of pathogenic bacterial of the *Enterobacteriaceae* group, such as *Enterobacter*, *Serratia*, *Escherichia*, *Proteus*, *Salmonella*, *Shigella*, *Klebsiella* (Diah et al., 2022), and *Citrobacter* (Fadli et al., 2021). In general, the cause of bleeding due to diarrheal infections is toxins produced by *Escherichia coli*, *Campylobacter* spp., *Salmonella spp.*, *Shigella spp.*, toxin-producing *Vibrio cholerae*, *Yersinia enterocolitica,* and *Citrobacter* spp. (Imran et al., 2020). *Escherichia coli* bacteria can quickly spread through water contamination and contaminated materials that come in contact with these bacteria (Hamidah et al., 2019). *Escherichia coli* is a gram-negative bacterium. It can be found in the intestine, indicating water contamination by fecal matter (Klau et al., 2021).

Diarrhea is a defecation frequency of more than three times a day, followed by loose fecal consistency with or without blood and mucus (Nurlaila & Susilawati, 2022).

Diarrhea is a major health problem in developing countries, such as Indonesia. According to the WHO (2000), there were around 4 billion cases of diarrhea in the world, and 2.2 million people died, mostly children under five years of age. Mostly, Indonesian regions from 2009 until now found that the incidence rate of diarrhea remains high, with around 162 thousand toddler deaths every year and approximately 460 thousand toddler deaths every day due to diarrhea (Novita, 2020).

Due to this information, an antibiotic is essential in gastrointestinal infection treatment, mainly caused by bacteria (Niken et al., 2023). However, irrational antibiotic use is still high, leading to bacterial resistance that decreases bacterial sensitivity (Damanik et al., 2021). Thus, looking for natural products with minimal side effects and preventing further bacterial resistance becomes important. Many herbs have been widely used in community settings as an alternative treatment for various diseases, especially diarrhea and other gastrointestinal infections (Salsabilla et al., 2023).

Indonesia is a tropical country with various plants that could become herbid. These herbs have fewer side effects and many health benefits. One of these plants, which has been reported to have various health benefits, is orange fruit. The sweet orange fruit has been used for a long time in the community, especially in Indonesia. However, most people utilize only the fruit flesh, and the orange peel is wasted as household waste (Filbert et al., 2023; Mutia & Manalu, 2020; Niken et al., 2023). Many nutrients and compounds, such as flavonoids, terpenoids, alkaloids, and essential oils, enrich the orange peel. These compounds potentially act as antibacterial agents that can inhibit bacterial growth and kill pathogenic bacteria by disrupting bacterial metabolism (Amiliah et al., 2021). Based on the research by Amiliah *et al.* (2021), the crude extract and essential oil of Kalamansi orange peel moderately inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* at a concentration of 40%. Amiliah et al. also reported that the essential oil of Kalamansi orange peel also reported to strongly inhibited the growth of *Staphylococcus aureus* and moderately inhibited the growth of *Escherichia coli* bacteria at a concentration of 20% (Amiliah et al., 2021). Another study by Niken (2023) also reported whether the antibacterial activity of orange peel extract positively correlated to its concentration. Twenty percent and a hundred of orange peel extract could inhibit the growth of *Staphylococcus aureus*, forming an inhibition zone diameter of 12.33 mm and 21.4 mm, respectively (Niken et al., 2023).

Based on the description above, looking for the antibacterial activity of orange peel as another pharmaceutical form against *Enterobacteriaceae* becomes important when the rate of water-borne gastrointestinal infection caused by bacteria is high. Thus, this study intends to investigate the antibacterial activity of sweet orange peels (*Citrus sinensis*) against *Enterobacteriaceae* isolated from a random water depot.

MATERIALS AND METHODS

Study Design

This experimental study was performed at Laboratorium Terpadu, Universitas Prima Indonesia, from April 2024 to June 2024. This study has also been approved by Komite Etik Penelitian Kesehatan (KEPK) Universitas Prima Indonesia with letter no. 016/KEPK/UNPRI/III/2024.

Materials

This experimental study used some material including Sweet orange peel, water sample from a random depot, chloramphenicol (paper disc antibiotic), Mueller Hinton Agar (MHA) media, Nutrient Agar Media, EMBA (Eosin Methylene Blue Agar), MacConkey Agar, distilled water, methanol solution, normal saline, 70% alcohol, iodine solution, crystal violet, safranin, acetone alcohol, lactose broth, NaOH, HCl, $FeCl_3$, Mayer reagent, Dragendroff reagent, Chloroform, anhydrous acetate acid, sulfur acid, $FeCl_3$ 5%

Isolation and Identification of Enterobacteriaceae from Water Depot Sample

Initially, this study was performed by isolating and identifying *Enterobacteriaceae* in a water sample from a random depot around the Faculty of Medicine, Dentistry, and Health Science, Universitas Prima Indonesia. After that, it was continued to evaluate the pharmaceutical formulation and antibacterial activity of sweet orange peel tea against the obtained *Enterobacteriaceae*.

Initially, this study selected randomized depot water to obtain a water sample and reserved it into a sealed bottle. Before the isolation and identification of *Enterobacteriaceae* in a water sample, this study prepared lactose broth, EMB agar, and MacConkey Agar. Lactose broth was made by dissolving 1.3 grams of lactose broth powder into ten milliliters of distilled water using a hot plate magnetic stirrer, and it was used for enrichment and serial dilution purposes. EMB agar was made by dissolving 1.6 grams of EMB agar into a hundred milliliters of distilled water using a hot plate magnetic stirrer, and it was used to subculture the bacterial-containing broth. MacConkey agar was made by dissolving 5.0 grams of MacConkey agar powder into a hundred milliliters of distilled water, and it was used to purify the colony from subculture media. Both agar and broth media were sterilized by autoclave 0.5 atm and 121°C for 15 minutes (N. Oktaviani et al., 2022; Toruan et al., 2023).

Isolation was begun by sample enrichment. Enrichment was performed by dissolving a milliliter of water sample into ten milliliters of lactose broth and

incubating it at 43°C for 24 hours (Yanestria et al., 2022). After that, a milliliter of incubated lactose broth was subcultured into EMB agar by spreader and incubated at 37°C for 20-24 hours. Then, the dominant colony was purified into MacConkey Agar several times until it obtained the homogenous colony in MacConkey Agar by the four-quadrant streak method (Diniarti et al., 2022; Yanestria et al., 2020).

The isolated *Enterobacteriaceae* from MacConkey agar was identified by biochemical assay. The IMViC test was used for the biochemical identification of Enterobacteriaceae, including Indole, Methyl Red, Voges Proskauer, Citrate, and Motility. IMViC test was performed using some reaction tubes.

Indole Test

Indole test was performed by inoculating the colony into Sulfide Indole Motility (SIM) media and incubating it for 24 hours at 37°C. After that, an Erlich reagent was added to the media to identify color change (Gunawan et al., 2022).

MR-VP Test

MR-VP test was performed using the same media in two different reaction tubes, which was MR-VP media in a similar way to the Indole test. After the incubation, it was dropped by methyl red for the MR Test and both alpha-naphthol and 40% KOH solution for the VP test (Sari et al., 2019)

Citrate test

The citrate test was performed by inoculating the colony into Simmons citrate medium, similar to other biochemical tests, and identifying the color changes after the incubation (Sari et al., 2019).

Motility Test

The motility test was performed similarly to the indole test by inoculating the colony within 1 cm of the bottom of the tube and incubating it for 24 hours at 37oC. After that, the bacteria's motion was identified around the inoculation line (Sari et al., 2019).

After the isolation and identification, An *Enterobacteriaceae*-containing MacConkey was reserved for further antibacterial assay.

Sweet Orange Peel Tea Formulation

This study then continued to formulate the orange peel tea. Sweet oranges were obtained from a modern Medan market and identified in Herbarium Medanense, Faculty of Mathematics and Science, Universitas Sumatera Utara. All obtained sweet oranges were sorted and peeled. Orange peel was collected and washed. After that, it was dried with a food dehydrator at 70°C for 24 hours (Acar et al., 2022). Afterward, the dried orange peel was ground by a blender and sieved with mesh number 40. Finally, this sieved orange simplicial was packed into 3-gram and 5-gram tea bags (Novitriani et al., 2021).

Both 3-gram and 5-gram tea bags were processed using two different methods: infusion and decoction. An infusion was performed by brewing both tea bags with a hundred milliliters of distilled water for fifteen minutes at 90°C. Meanwhile, a decoction was performed in the same way, but for a longer time: thirty minutes (Chiuman et al., 2021). Finally, both infusion and decoction of orange peel tea underwent a phytochemical screening to identify the phytochemicals, including flavonoid, tannin, alkaloid, steroid/triterpenoid, phenol, and saponin (Gulo et al., 2021; Suhartomi et al., 2020; Sumartin et al., 2024).

Antibacterial Assay

This study continued to investigate the antibacterial activity from infusion and decoction of orange peel tea against *Enterobacteriaceae* isolated from a random water depot by disc diffusion method. Initially, this study prepared some media that would be used, including Nutrient Agar (NA) and Mueller Hinton Agar (MHA). Nutrient Agar (NA) was made by dissolving 2.8 grams of NA powder into a hundred milliliters of distilled water using a hot plate magnetic stirrer. Meanwhile, MHA was made by dissolving 3.8 grams of a hundred distilled water using a hot plate magnetic stirrer. Both media were sterilized by an autoclave at 121°C for 15 minutes. After sterilizing both media, they were poured into some petri dish and chilled. After these media became solid, all Petri dishes were reserved in a refrigerator until these media were used (Amiliah et al., 2021; Damanik et al., 2021; Mutia et al., 2021)

Enterobacteriaceae isolates in the reserved MacConkey was rejuvenated in Nutrient Agar before being used in the disc diffusion method. An inoculum of *Enterobacteriaceae* from MacConkey agar were streaked by a quadrant streak method and incubated for 24 hours at 37°C (Daud et al., 2023). After bacterial rejuvenation, it was continued to suspend an *Enterobacteriaceae* colony from NA into the standard saline solution and formed a bacterial suspension with a bacterial concentration equivalent to 0.5 McFarland scale. An inoculum of Enterobacteriaceae colony from NA was suspended into ten milliliters of normal saline in the reaction tube and then homogenized by a vortex. Bacterial suspension concentration was measured based on the turbidity of the solution by UV-Vis Spectrophotometer at a wavelength of 600 nm (Amiliah et al., 2021; Komara et al., 2022).

Bacterial suspension and MHA media, prepared before, were used for disc diffusion. Every petri dish that contains MHA agar has a capacity of four test discs. *Enterobacteriaceae* isolate was streaked into sterilized MHA with a sterile cotton swab. After that, diffused discs that had been soaked in either infusion orange peel tea or decoction orange peel tea at any dose were placed on the surface of the MHA agar. A similar treatment was

performed in the standard and control groups. Standard and control groups used chloramphenicol-containing discs and distilled water-diffused discs, respectively. Moreover, all these petri dishes were incubated at 37°C for 24 hours. After the incubation, the formed clear zone was measured as an inhibition zone diameter and expressed in millimeters by caliper (Chiuman et al., 2023; Damanik et al., 2021; Jungjunan et al., 2023; Utami et al., 2021).

Data Analysis

All data in this study was analyzed by IBM SPSS Statistic 27. Initially, the determination of orange fruit, phytochemical screening, and inhibition zone diameter were analyzed by descriptive statistics. After that, the diameter of the inhibition zone was analyzed using inferential statistics, Kruskal-Wallis.

RESULTS AND DISCUSSION

This experimental laboratory study aimed to investigate the antibacterial activity of sweet orange peel tea against *Enterobacteriaceae,* which was isolated from random water depots around Universitas Prima Indonesia by the disc diffusion method. The sweet oranges were obtained from one of the modern markets in Medan, North Sumatra, formulated into tea, and brewed using two different methods: infusion and decoction. This study demonstrated the antibacterial activity from infusion and decoction of tea orange peels, except for the formulation of 3-gram orange peel tea brewed by infusion.

Initially, this study isolated and identification of *Enterobacteriaceae* from a random water depot sample around Universitas Prima Indonesia. This study used Eosin Methylene Blue Agar to isolate bacteria from Water Depots, and Figure 1 presents the isolated *Enterobacteriaceae* in EMB agar.

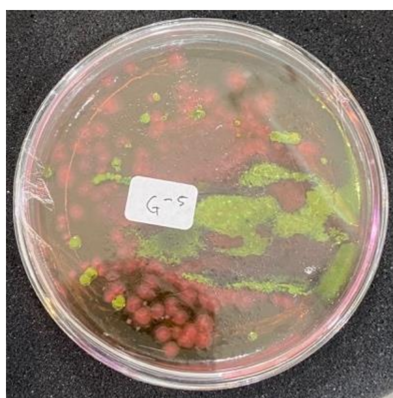

Figure 1. Isolate Bacterial on Eosin Methylene Blue (EMB) Agar.

Isolated bacteria on EMB Agar show metallic green sheen colonies and are suspected to be *Escherichia coli.* This study used EMB agar due to the selectivity and differentiation of this medium for the growth of enteric bacteria and coliform microorganisms. This media is rich in nutrients to support the growth of *Enterobacteriaceae*, especially lactose fermenter bacteria (Putri et al., 2023; Salaila et al., 2024). Furthermore, EMB also has eosin, which inhibits the growth of positive-gram bacteria and enhances the growth of negative-gram bacteria, such as *Escherichia coli* (Akhnah et al., 2022). On the other hand, EMB also has lactose as a substrate for lactose fermenter bacteria, then eosin and methylene blue in this media were used to differentiate either lactose or non-lactose fermenter bacteria by media color change (Hendiana et al., 2022). A dark color with a shiny metal colony indicates a lactose fermenter bacteria. Brown and pink color colonies indicate a slow lactose fermenter bacteria. Finally, the Faint red color colony indicated a non-lactose fermenter bacteria (Akhnah et al., 2022; Hendiana et al., 2022).

This study showed that the isolated bacteria from a random water sample depot had a greenish metallic sheen color, indicating lactose fermenter bacteria. This color changes due to the products of some acid compounds from lactose fermentation. This acid compound reacted with methylene blue to form a black or dark color and, with a metallic green center, separate colonies with a diameter of 2-3mm (Bollyn et al., 2023; Yanestria et al., 2020). Furthermore, bacteria with green sheen metallic colonies were purified on MacConkey Agar medium three times. The results of bacteria purification on MacConkey Agar can be seen in Figure 2.

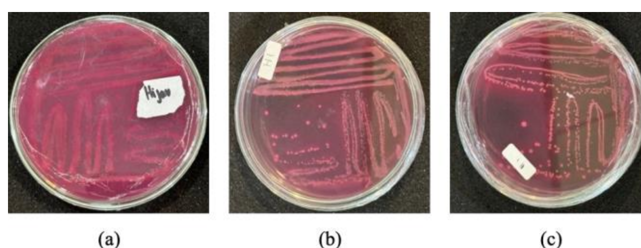

Figure 2. The Purification of Isolate Bacteria on MacConkey Agar (a) First purification (b) Second purification (c) Third purification.

Figure 2 shows that purified bacteria in MacConkey Agar had pink-colored colonies, which indicates the ability to lactose the fermenter. MacConkey was a differential media for either lactose fermenter or non-lactose fermenter bacteria. A pink color colony indicated a lactose fermenter bacteria due to the formation of some acid compounds as a result of a lactose fermentation reaction ($pH < 6.8$) (Nursanty et al., 2019). This study showed that the colony was pink, indicating lactose fermenter bacteria. Some *Enterobacteriaceae* with lactose fermentation activity include *Escherichia coli*, *Klebsiella sp., Enterobacter aerogenes*, and *Citrobacter sp* (Akhnah et al., 2022). There are some lactose fermenter *Enterobacteriaceae*; Therefore, additional tests are required to identify the type of *Enterobacteriaceae*. Due to this reason, the obtained colony underwent some biochemical tests (Lisdewi et al., 2023). After

purification, a colony from each Petri was stained with gram staining, as described in Figure 3.

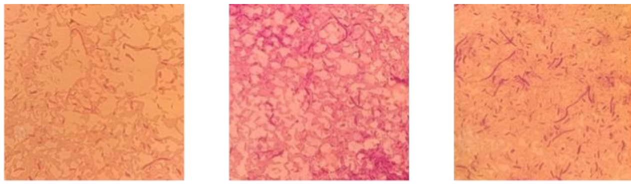

Figure 3. The Results of Gram Staining under a Microscope with 1000x Magnification of Enterobacteriaceae Isolate Bacteria.

Figure 3 shows that these colonies have negative-gram staining bacteria, a rod red-staining bacterium. It indicated a rod-shaped *Enterobacteriaceae*. Isolated bacteria cannot retain crystal violet as a primer stain after being flushed by a decolorizing agent of alcohol or acetone. Hence, this bacteria absorbs the counterstain, safranin (red color). A thin layer of negative-gram bacteria cell wall causes this bacteria to be unable to retain the primer color. Hence, the lipid layer of the negative-gram bacteria cell wall was dissolved by the decolorizing agent (alcohol or acetone) during the gram staining process (Rahmatullah et al., 2021; Sari et al., 2020)

Gram-negative bacteria cell wall consists of phospholipids, lipopolysaccharides, and lipoproteins. These layers cause the impermeable property of bacteria cell walls for small molecules. It also causes an obstacle for the antibacterial substance to pass the negative-gram bacterial cell wall. Another factor affecting the permeability of negative-gram bacteria was porins, which were part of the bacterial protein. Due to these reasons, some large molecular sizes of antibacterial are challenging to penetrate the negative-gram bacteria and lead to more resistant antibiotic properties (Samputri et al., 2020).

According to EMB Agar, MAC agar, and gram staining, the obtained isolated bacteria from a random water sample depot may be a lactose fermenter *Enterobacteriaceae,* including *Escherichia coli*, *Citrobacter sp.*, *Klebsiella sp.*, or *Enterobacter aerogenes*. A biochemical test was required to identify the possible type of *Enterobacteriaceae* (Akhnah et al., 2022). Thus, a colony from these MacConkey Agars underwent a biochemical test, including: IMViC and motility tests were performed to identify *Enterobacteriaceae* bacteria isolated from random Water Depots around Universitas Prima Indonesia, and the result of the biochemical test is described in Table 1.

Table 1. Biochemical Test Results of *Enterobacteriaceae* Isolate Bacteria.

Biochemical Test	**Reagent**	**Results**
Indole Test	Reagent Erlich	-
Methyl Red Test	Methyl Red Indicator	+ (weak positive)
Voges-Prosakeur Test	Alpha-naphtol + KOH 40%	-
Sitrat Test	Media Simmon Citrate	+
Motility Test	Media Sulfide Indole Motility (SIM)	+

Table 1 shows the colony's cheerful methyl red, citrate, and motility tests. It can be concluded that isolated *Enterobacteriaceae* could convert pyruvic into a stable acid compound (MR Test) and utility citrate as a carbon source into oxaloacetic acid and acetic acid (Citrate test).

The Methyl Red Test (MR Test) is a biochemical test to determine the ability of bacteria to convert pyruvate from a glycolysis reaction into an acid compound. The methyl red test uses a broth media that consists of glucose as a substrate for glycolysis, and then the bacteria uses glucose for glycolysis and produces some pyruvate compounds. Some bacteria may convert these pyruvate compounds into stable acid compounds. This acid compound was identified by the reaction of acid into methyl red reagent. Hence, the positive result was identified as red, and the negative test result as yellow. This study showed that the isolated *Enterobacteriaceae* from a random water depot sample had a red color from the methyl red test, which indicated a positive result. It showed that isolated *Enterobacteriaceae* could convert pyruvate into a stable acid compound (Lisdewi et al., 2023).

This isolated bacteria from a random water depot also showed an ability to use citrate as a carbon source for oxaloacetic acid and acetic acid for energy formation. It can be seen from the simmon citrate test, which uses simmon citrate media. Simmon citrate media contains some compounds, including citrate as a substrate for citrate enzyme, ammonium ion as a nitrogen source, and the last one is bromothymol blue (BTB) as a pH indicator. Some bacteria can utilize citrate as a carbon source, and it will break the citrate compound into oxaloacetic acid and acetic acid. The oxaloacetic acid will then be decarboxylated to produce pyruvate and carbon dioxide gas. After that, carbon dioxide gas will react with water molecules and excess sodium citrate in simmon citrate media to produce sodium carbonate that has alkaline properties. After that, the high release of carbon dioxide from the last reaction will induce the ammonium salt metabolism to produce ammonium hydroxide. Both ammonium hydroxide and sodium

carbonate increase the pH in alkaline conditions. Thus, it turns the BTB as a pH indicator into a Prussian blue. This study showed that the isolated *Enterobacteriaceae* changed the slant agar into a Prussian blue color that indicated a positive citrate test, and it showed that the isolate *Enterobacteriaceae* used the citrate as a source of carbon and ammonium salts as the nitrogen source (Zebua et al., 2020) (Apriyanthi et al., 2022) (Sari et al., 2019). The last biochemical test observed in this study was the motility test. Isolated Enterobacteriaceae from a random water depot sample was a motile bacteria. It can be seen from the growth pattern of bacteria in SIM (Sulfide-Indole-Motility) media, which spread out from the medium site. Some *Enterobacteriaceae* may have a propeller-like flagella or unique fibrils that produce a gliding form of motility.

Based on the macroscopic (EMB agar and MacConkey), microscopic (gram-staining), and biochemical tests (IMViC and motility), isolated *Enterobacteriaceae* from water depot sample preferred to *Citrobacter sp*. Some previous studies have reported the presence of various types of *Enterobacteriaceae* from either source, such as fish, water, et cetera. Initially, this study identified the isolated bacteria from the macroscopic appearance of colonies in EMB and MacConkey agar, which was in line with the result of Kim et al. in 2015. Kim et al. reported that both *Citrobacter braakii* and *Escherichia coli* on MCA and Levine's Eosin Methylene Blue Agar (L-EMB) had an identical *Enterobacteriaceae*, which indicates that *Citrobacter braakii* can ferment lactose similar to *Escherichia coli*. Due to this reason, it can be concluded that the probable isolated *Enterobacteriaceae* from random water depot was either *Citrobacter sp.* or *Escherichia coli* (Kim et al., 2015). The obtained *Enterobacteriaceae* from a random water depot around Universitas Prima Indonesia also showed some biochemical characteristics that could be used to investigate *Enterobacteriaceae* type. Warpala et al. (2015) reported that isolated *Citrobacter sp.* showed a positive MR and Citrate test in the biochemical test from isolated *Enterobacteriaceae* from Buyan Lake's water sample. *Citrobacter sp.* and *Escherichia coli* shared a positive MR test. Both indole and citrate tests can be helpful in differentiating *Citrobacter sp.* and *Escherichia coli*. *Citrobacter sp.* utilites citrate as a carbon source for energy formation, which is not found in *Escherichia coli* and leads to a positive citrate test. When *Escherichia coli* hydrolyzes tryptophan to form indole, it leads to a positive indole test. These previous studies showed a similar result to the current study, which reported that the isolated *Enterobacteriaceae* from a random water depot sample around Universitas Prima Indonesia was *Citrobacter sp.* with positive MR Test and Citrate test results (Warpala et al., 2019). Moreover, Fadli et al. (2021) also reported that the refill water depot sample in Jambi has some *Enterobacteriaceae,* including *Klebsiella Sp.* and *Citrobacter sp.*, which were identified from macroscopic, microscopic, and biochemical test characteristics (Fadli et al., 2021).

This study was then continued to investigate the antibacterial activity of sweet orange peel tea against the *Enterobacteriaceae* isolated from the water depot sample. Sweet orange fruit samples were obtained from one of the modern markets in Medan, North Sumatra. The sweet orange fruit was then identified, and its taxonomy was described below.

Kingdom : *Plantae*
Divisi : *Spermatophyta*
Class : *Dicotyledoneae*
Ordo : *Sapindales*
Family : *Rutaceae*
Genus : *Citrus*
Species : *Citrus sinensis* (*Burm.*) *Merr.*
Local Names : Sweet Orange

Phytochemical screening in this study was performed to determine the presence of secondary metabolites found in sweet orange (*Citrus sinensis*) peel tea. In this study, the results of the phytochemical screening test on sweet orange peel tea were described in Table 2 and Table 3.

Table 2. Screening phytochemical results of sweet orange peel tea infusion.

Secondary Metabolites Compounds	Reagent	Result
Flavonoid	NaOH + HCl	+
Tannin	FeCl3	+
Alkaloid	Mayer	-
	Dragendorff	+
Saponin	HCl	-
Steroid	Chlorofom + anhydrous acetate acid + sulfur acid	-
Triterpenoid	Chlorofom, anhydrous acetate acid, sulfur acid	+
Phenol	FeCl3 5%	+

Table 3 Screening phytochemical results of sweet orange peel tea decoction.

Secondary Metabolites Compounds	Reagent	Result
Flavonoid	NaOH + HCl	+
Tannin	$FeCl_3$	+
Alkaloid	Mayer	-
	Dragendorff	+
Saponin	HCl	-
Steroid	Chloroform + anhydrous acetate acid + sulfur acid	-
Triterpenoid	Chloroform + anhydrous acetate acid + sulfur acid	+
Phenol	$FeCl_3$ 5%	+

According to the phytochemicals screening result, Sweet orange peel infusion or decoction has some phytochemicals, including flavonoids, tannins, alkaloids, triterpenoids, and phenol. Then, it was continued to antibacterial assay by disc diffusion method, and the results of measuring the antibacterial activity of sweet orange peel tea against *Enterobacteriaceae* bacteria isolates through the diameter of the bacterial inhibition zone observed for 24 hours. Inhibition zone diameter data was initially analyzed using Shapiro-Wilk for data distribution, and the result of Shapiro-Wilk was described in Table 4.

Table 4. The results of Shapiro-Wilk Analysis for data distribution.

Group	*P-Value*	Distribution
3g Infusion	0.720	Normal
3g Decoction	0.000	Not Normal
5g Infusion	0.843	Normal
5g Decoction	0.949	Normal
Standard (Chloramphenicol)	0.092	Normal
Control (Distilled Water)	0.000	Not Normal

Based on shapiro-wilk analysis, the inhibition zone diameter of 3 grams decoction (P-Value: 0.000) and control (P-Value: 0.000) has a non-normal data distribution. Hence, it can be concluded that the data distribution of inhibition zone diameter was not expected. For this reason, this study compared the inhibition zone diameter by Kruskal-Wallis, which is described in Table 5.

Table 5. Comparison of Inhibition Zone Diameter in All Tea Formulation Group.

Tea Formulation	Inhibition Zone Diameter (mm)			Mean	Std. Deviation	P-Value
	1st repetition	2nd repetition	3rd repetition			
3g Infusion	2.51	2.86	1.92	2.43	0.45	0.010
3g Decoction	0.00	0.00	0.00	0.00	0.00	
5g Infusion	2.51	2.86	2.71	2.71	0.17	
5g Decoction	2.31	3.66	5.14	3.70	1.41	
Standard	26.71	26.81	28.56	27.36	1.04	
Control	0.00	0.00	0.00	0.00	0.00	

It can be concluded that there was a significant difference in inhibition zone diameter among all tea formulation groups; it can be seen from P-Value < 0.05 (P-Value: 0.010). The highest mean was found in the standard group that received chloramphenicol, followed by 5 grams decoction, 5 grams infusion, 3 grams infusion, and 3 grams decoction. Furthermore, this study analyzed inhibition zone diameter in each group using the Mann-Whitney test, and the analyses results are described in Table 6.

Table 6. The results of the Whitney test.

Group	Comparison	P-Value	Interpretation
Control	3g Infusion	0.037*	Significant
	3g Decoction	1.00	Not Significant
	5g Infusion	0.037*	Significant
	5g Decoction	0.037*	Significant
	Standard	0.037*	Significant
Standard	3g Infusion	0.050*	Significant
	3g Decoction	0.037*	Significant
	5g Infusion	0.050*	Significant
	5g Decoction	0.050*	Significant
5g Decoction	3g Infusion	0.275	Not Significant
	3g Decoction	0.037*	Significant
	5g Infusion	0.513	Not Significant
5g Infusion	3g Infusion	0.500	Not Significant
	3g Decoction	0.037*	Significant
3g Decoction	3g Infusion	0.037*	Significant

Table 6 showed that the control (distilled water) and the formulation of 3-gram orange peel decoction did not show any significant difference in inhibition zone diameter (P-Value > 0.05). However, the inhibition zone diameter of distilled water significantly differed from other groups (3 grams orange peel infusion, 5 grams decoction and infusion, and standard group). Moreover, the changes in orange peel simplicial mass in formulation significantly affected the antibacterial activity against the isolated *Enterobacteriaceae*. However, the method of brewing did not affect the antibacterial activity.

The antibacterial assay showed that either orange peel tea infusion or decoction had antibacterial activity against isolated *Enterobacteriaceae* from a random water depot sample. It can be seen from the mean of the inhibition zone from 3-gram infusion, 5-gram infusion, and 5-gram decoction, which were 2.43 mm, 2.71 mm, and 3.70 mm. Only 3-gram decoction did not show an antibacterial assay. This antibacterial activity of orange peel tea comes from phytochemicals, including flavonoid, tannin, alkaloid, triterpenoid and phenol compounds found in either infusion or decoction. These phytochemicals were in line with Erika et al. (2023), who reported that sweet orange peel extract (*Citrus sinensis* (L.)) contained flavonoids, saponins, tannins, and essential oils (E. D. Oktaviani et al., 2023). These phytochemicals are reported to have both antibacterial and antioxidant activity. Flavonoid was reported to disturb membrane cells, microsomes, and lysosomes by forming a complex compound with this membrane and leading to organelle efflux. When flavonoid binds to the bacterial wall's outer membrane, it disturbs the bacterial wall's stability, leads to cytoplasm leaks, inhibits bacterial growth, and ends in bacterial death. Flavonoids are also reported to bind bacterial DNA and disturb its structure. Other phytochemicals such as tannin, saponin, and triterpenoid disturbed cell membrane stability. Tannin can also bind to intracellular bacterial proteins and precipitate these proteins, which causes internal structure damage (Niken et al., 2023) (Amiliah et al., 2021)(Egra et al., 2019).

This study also compared the antibacterial activity of orange peel tea formulation to chloramphenicol as standard and distilled water as control. Chloramphenicol is a broad-spectrum antibiotic for positive and negative gram bacteria (Aviany & Pujiyanto, 2020). Chloramphenicol is an antibiotic that inhibits bacterial ribosomes and inhibits bacterial protein synthesis. Tannin in orange peel tea showed a similar mechanism of action, precipitating intracellular bacterial protein. On the other hand, this study also used distilled water as a control for tea solvent, and it did not have any antibacterial effect due to the absence of an inhibition zone in the antibacterial assay (Azizah & Antarti, 2019).

CONCLUSIONS

It can be concluded that sweet orange peel tea infusion and decoction had weak antibacterial activity against the Enterobacteriaceae isolated from random water depot samples around Universitas Prima Indonesia (P value: 0.010). These isolated Enterobacteriaceae tended to be Citrobacter sp. This weak antibacterial activity was shown by the mean of 3 gram infusion, 5 gram infusion and 5 gram decoction, which were 2.43 mm, 2.71 mm and 3.70 mm, respectively.

Acknowledgements: All the authors would like to thank all those who supported this research process.

Authors' Contributions:
Conceptualization: Yumiko, Suhartomi, and Sri Wahyuni; **Methodology:** Siti Syarifah and Ade Pryta R. Simaremare; **Investigation:** Yumiko, Suhartomi, and Sri Wahyuni; **Discussion of results:** Yumiko and Suhartomi; **Writing – Original Draft:** Yumiko and Suhartomi; **Writing – Review and Editing:** Sri Wahyuni, Siti Syarifah, and Ade Pryta R. Simaremare; **Supervision:** Suhartomi and Sri Wahyuni; **Approval of the final text:** Suhartomi, Siti Syarifah, and Sri Wahyuni.

Competing Interests: All authors declare no competing interests.

Funding: This study did not receive any funding.

REFERENCES

Acar, A., Aydın, M., & Arslan, D. (2022). Development of infusion tea formulations with food wastes: Evaluation of temperature and time effects on quality parameters. *Applied Food Research*, *2*(1).

Akhnah, A. M., Widyastuti, D. A., & Rachmawati, R. C. (2022). Identifikasi Genera Bakteri Coliform Pada Air Sungai Desa Datar Kabupaten Jepara. *Quagga: Jurnal Pendidikan Dan Biologi*, *14*(2), 124–131. https://doi.org/10.25134/quagga.v14i2.5061

Amiliah, Nurhamidah, & Handayani, D. (2021). Aktivitas Antibakteri Kulit Buah Jeruk Kalamansi (Citrofortunella Microcarpa) Terhadap Bakteri Staphylococcus aureus dan Escherichia coli. *Jurnal Pendidikan Dan Ilmu Kimia*, *5*(1), 92–105. https://doi.org/10.33369/atp.v5i1.16493

Apriyanthi, D. P. R. V., Laksmita, A. S., & Widayanti, N. P. (2022). Identifikasi Bakteri Kontaminasi pada Gelang Tri Datu. *Jurnal Biologi Makassar*, *7*(2), 24–33. https://journal.unhas.ac.id/index.php/bioma

Aviany, H. B., & Pujiyanto, S. (2020). Analisis Efektivitas Probiotik di dalam Produk Kecantikan sebagai Antibakteri terhadap Bakteri Staphylococcus epidermidis. *Jurnal Berkala Bioteknologi*, *3*(2), 24–31.

Azizah, R., & Antarti, A. N. (2019). Uji Aktivitas Antibakteri Ekstrak Dan Getah Pelepah Serta Bonggol Pisang Kepok

Kuning (Musa paradisiaca Linn.) Terhadap Bakteri Pseudomonas aeruginosa dan Klebsiella pneumoniae Dengan Metode Difusi Agar. *JPSCR: Journal of Pharmaceutical Science and Clinical Research*, *4*(1), 29. https://doi.org/10.20961/jpscr.v4i1.26544

Bollyn, Y. M. F., Gultom, R., Luju, M. T., Rinca, K. F., & Achmadi, P. C. (2023). *Identifikasi Cemaran Eschericia coli pada Daging Babi Segar di Pasar Ruteng, Nusa Tenggara Timur*. *22*, 167–173.

Chiuman, L., Ginting, C. N., Yulizal, O., Suhartomi, & Chiuman, V. (2021). Improvement of Liver Function from Lemon Pepper Fruit Ethanol Extract in Streptozotocin-Induced Wistar Rats. *2021 IEEE International Conference on Health, Instrumentation & Measurement, and Natural Sciences (InHeNce)*. https://doi.org/10.1109/InHeNce52833.2021.9537284

Chiuman, L., Sherlyn, Aritonang, N. S., Rudy, & Suhartomi. (2023). In Vitro Study of Antibacterial Activity of Snake Fruit Extract against Extended Spectrum Beta Lactamase (ESBL) Escherichia coli. *Jurnal Aisyah: Jurnal Ilmu Kesehatan*, *8*(2), 715–720. https://doi.org/10.30604/jika.v8i2.1962

Damanik, D. F., Monica, Lubis, Y. M., & Meldawati. (2021). *Uji Aktivitas Anti bakteri Ekstrak Lidah Buaya (Aloe vera) terhadap Bakteri Pseudomonas aeruginosa dan Streptococcus pyogenes*. *44*(6), 357–364.

Daud, N. S., Arni, D. P., Idris, S. A., & Saehu, M. S. (2023). Uji Aktivitas Antibakteri Ekstrak Batang Meistera chinensis Terhadap Escherichia coli ATCC 35218. *Warta Farmasi*, *12*(1), 8–18.

Diah, H., Ervan, & Riyadi, A. (2022). Enterobacteriaceae dan Personel Hygiene Ibu Terhadap Kejadian Diare Anak 5 Tahun di Kecamatan Ratu Samban Kota Bengkulu. *Journal of Pharmaceutical and Health Research*, *3*(3), 78–81. https://doi.org/10.47065/jharma.v3i3.2769

Diniarti, F. A., Kasasiah, A., & Hilmi, I. L. (2022). *Uji Resistensi Escherichia coli Dari Sumber Air Baku DI Karawang Terhadap Antibiotik Siprofloksasin*. *4*(3).

Egra, S., Mardhiana,., Rofin, M., Adiwena, M., Jannah, N., Kuspradini, H., & Mitsunaga, T. (2019). Aktivitas Antimikroba Ekstrak Bakau (Rhizophora mucronata) dalam Menghambat Pertumbuhan Ralstonia Solanacearum Penyebab Penyakit Layu. *Agrovigor: Jurnal Agroekoteknologi*, *12*(1), 26. https://doi.org/10.21107/agrovigor.v12i1.5143

Fadli, M., Hanina, Halim, R., Wulandari, P. S., & Hz, T. W. E. (2021). Identifikasi Genus Bakteri Klebsiella Dan Citrobacter Hasil Isolasi Dari Air Minum Isi Ulang Kota Jambi. *Jambi Medical Journal*, *Spescial*, 418–427.

Filbert, K., Wijaya, S., Budi, A., & Tobing, A. N. L. (2023). *Uji Aktivitas Antibakteri Ekstrak Kulit Jeruk Bali (Citrus Maxima Pericarpium) Terhadap Pseudomonas dan Enterococcus Faecalis*.

Gulo, K. N., Suhartomi, Saragih, A. D., Raif, M. A., & Ikhtiari, R. (2021). Antioxidant Activity of Flavonoid Compounds in Ethanol and Ethyl Acetate Extract from Citrus Sinensis. *AIMS 2021 - International Conference on Artificial Intelligence and Mechatronics Systems*, 1–6. https://doi.org/10.1109/AIMS52415.2021.9466078

Gunawan, Kholik, & Agustin, A. L. D. (2022). Profil Uji Biokima Hasil Isolasi Escherichia coli pada Feses, Air Minum Dan Air Saluran Buangan Kandang Sapi Bali di Kelompok Tani Ternak Menemeng (KT2M) Kabupaten Lombok Tengah Profile. *Mandalika Veterinary Journal*, *2*(1), 26–36.

Hamidah, M. N., Rianingsih, L., & Romadhon. (2019). Aktivitas Antibakteri Isolat Bakteri Asam Laktat Dari Peda dengan Jenis Ikan Berbeda Terhadap E. coli Dan S.aureus. *Ilmu Dan Teknologi Perikanan*, *1*(2).

Hendiana, S. N. A., Kasasiah, A., & Utami, M. R. (2022). Uji Cemara Escherichia coli pada Jamu Gendong dengan Metode Most Probable Number. *J. Sains Dan Teknologi Pangan*, *7*(4), 5375–5386.

Imran, A. Z. K., Ali, A. J. M., & Shareef, H. K. (2020). Isolation and molecular identification of Citrobacter freundii from diarrheal patient in Babylon Province, Iraq. *Plant Archives*, *20*(January), 2861–2865.

Jungjunan, R. A., Rahayu, P., Yulyuswarni, & Ardini, D. (2023). Antibacterial Activity and Effectiveness test of Bandotan (Ageratum conyzoides Linn.) Leaves Ethanol Extract Against Staphylococcus aureus. *Jurnal Analis Farmasi*, *8*(1), 13–32.

Kim, H. S., Kim, Y. J., Chon, J. W., Kim, D. H., Kim, K. Y., & Seo, K. H. (2015). Citrobacter braakii: A Major Cause of False-Positive Results on MacConkey and Levine's Eosin Methylene Blue Selective Agars Used for the Isolation of Escherichia Coli from Fresh Vegetable Samples. *Journal of Food Safety*, *36*(1), 33–37. https://doi.org/10.1111/jfs.12210

Klau, M. L. C., Indriarini, D., & Nurina, R. L. (2021). Uji Aktivitas Ekstrak Etanol Daun Kemangi (Ocimum Sanctrum L.) Terhadap Pertumbuhan Bakteri Escherichia coli Secara In VItro. In *Uji Aktivitas Ekstrak Etanol Cendana Medical Journal* (Vol. 21, Issue 1).

Komara, D., Turnip, M., & Kurniatuhadi, R. (2022). Potensi Uji Daya Hambat Bakteri Asam Laktat Isolat Lactobacillus sp. (KG61) terhadap Escherichia coli dan Staphylococcus aureus. *Agroprimatech*, *6*(1), 25–31.

Lisdewi, A., Kallau, N. H. G., & Detha, A. I. R. (2023). Detection Of Antibiotics Resistant Escherichia coli in Water Sources from Poultry Farming Environments In Kelapa Lima District, Kupang City. *Jurnal Veteriner Nusantara*, *6*(23), 1–14. http://ejurnal.undana.ac.id/jvn

Mutia, M. S., Annisa, E., & Suhartomi. (2021). Anti-Bacterial Activity of Ethanol Extract of Indian Borage (Coleus Amboinicus) Leaves against Bacillus Cereus. *Healthy Tadulako Journal (Jurnal Kesehatan Tadulako)*, *7*(1), 30–34. https://doi.org/10.22487/htj.v7i1.151

Mutia, M. S., & Manalu, C. V. (2020). Uji Efektivitas Ekstrak Etanol Kulit Jeruk Manis (Citrus Sinensis) Terhadap Bakteri Vibrio Cholerae. *(Jurnal Ilmiah Mahasiswa Kesehatan Masyarakat)*, *5*(1), 6–9.

Niken, Arman, E., Pebriansyah, R., & Yusuf, R. N. (2023). Uji Efektivitas Antibakteri Ekstrak Kulit Jeruk Manis (Citrus Sinensis) Terhadap Pertumbuhan Bakteri Staphylococcus Aureus. *Jurnal Kesehatan Saintika Meditory*, *6*(2), 296–305.

Novita, O. T. (2020). Hubungan Pola Asuh Orang Tua Dengan Kejadian Diare Pada Balita di Puskesmas Mergangsan Kota Yogyakarta. *Journal Of Dehasen Educational Review*, *1*(2), 56–64.

Novitriani, K., Suhartati, R., Gusnanda, A., & Sifa, N. I. A. (2021). *Efektifitas Herbal Kemasan Celup dalam MenghambatvPertumbuhan Bakteri Escherichia coli*. *1*, 21–28.

Nurlaila, N., & Susilawati, S. (2022). Pengaruh kesehatan lingkungan terhadap kejadian diare pada balita di Kota Medan. *Nautical: Jurnal Ilmiah Multidisiplin*, *1*(6), 463–466. https://jurnal.arkainstitute.co.id/index.php/nautical/article/view/389%0Ahttps://jurnal.arkainstitute.co.id/index.php/nautical/article/download/389/319

Nursanty, Ri., Sari, Wi., & Safranita. (2019). Karakterisasi dan Identifikasi Bakteri Enterobacteriaceae pada Telur Penyu Lekang (Lepidochelys olivacea) asal Lhok Pante Tibang, Banda Aceh. *Jurnal Sain Veteriner*, *37*(1), 41–48. https://doi.org/10.22146/jsv.48514

Oktaviani, E. D., Sofiah, Oktriyanti, M., Hartati, N., Taufik, M., & Anggriani, I. U. (2023). Produk Gel Hand Sanitizer sebagai Antiseptik dari Ekstrak Kulit Jeruk Manis (Citrus sinensis (L.)). *Journal Education and Chemistry*, *5*(2), 93–99.

Oktaviani, N., Sulistiyawati, I., & Rahayu, N. L. (2022). Isolasi dan Karakterisasi Umum Mikroba yang Diduga Enterobacteriaceae pada Jajanan di Wilayah Purwokerto Menggunakan Medium EMBA. *Scientific Reports*, *2*, 41–51.

Parawidnyaningsih, P. A. A., Masfufatun, M., Listyawati, A. F., Kuntaman, K., & Sudibya, A. (2023). Detection of Enterobacteriaceae Lactose Fermenter Bacteria Producing Extended Spectrum Beta-Lactamase (ESBL) in Food Samples at Surabaya. *Bioma : Jurnal Ilmiah Biologi*, *12*(2), 38–45. https://doi.org/10.26877/bioma.v12i2.16618

Putri, M. K., Oktari, A., Oktiansyah, R., & Ulandari, T. (2023). Uji Cemaran Salmonella sp. dan Eshcerichia coli Pada Makanan Olahan Daging Ikan GIling. *Maximus: Journal of Biological and Life Science*, *1*(1), 10–14. https://journal.itera.ac.id/index.php/maximus/article/view/1151%0Ahttps://journal.itera.ac.id/index.php/maximus/article/download/1151/372

Rahmatullah, W., Novianti, E., & Dewi, A. L. S. (2021). Identifikasi Bakteri Udara Menggunakan Teknik Pewarnaan Gram. *Jurnal Ilmu Kesehatan Bhakti Setya Medika*, *6*(2), 83–91. https://www.poltekkes-bsi.ac.id/jurnal/index.php/bsm/article/download/62/37/

Riedel, S., Morse, S. A., Mietzner, T., & Miller, S. (2019). *Jawetz, Melnick & Adelberg's medical microbiology*.

Salaila, T. J., Tumbel, F. M., Lawalata, H. J., Tanorl, M. N., & ... (2024). Identification of Escherichia Coli Bacteria in Tofu Soaking Water Sold at Bersehati Market Manado City North Sulawesi Province. *Jurnal Pendidikan ...*, *8*, 17709–17721. https://jptam.org/index.php/jptam/article/view/14900%0Ahttps://jptam.org/index.php/jptam/article/download/14900/11364

Salsabilla, W., Saula, L. S., & Sholih, M. G. (2023). Uji Aktivitas Antidiare Ekstrak Etanol Kulit Jeruk Lemon (Citrus Limon (L.) Burm. F.) dan Daun Jambu Biji (Psidium Guajava L) Pada Mencit Jantan Galur Swiss Webster. *Jurnal Bidang Ilmu Kesehatan*, *13*(1), 51–58.

Samputri, R. D., Toemon, A. N., & Widayati, R. (2020). Uji Aktivitas Antibakteri Ekstrak Etanol Biji Kamandrah (Croton tilgium L.) Terhadap Pertumbuhan Salmonella typhi Dengan Metode Difusi Cakram (Kirby-Bauer). *Herb-Medicine Journal*, *3*(3), 19. https://doi.org/10.30595/hmj.v3i3.6393

Sari, D. P., Amir, H., & Elvia, R. (2020). Isolasi Bakteri dari Tanah Tempat Pembangan Akhir (TPA) Air Sebakul sebagai Agen Biiodegradasi Limbah Plastik Polyethylene. *Jurnal Pendidikan Dan Ilmu Kimia*, *4*(2), 98–106.

Sari, D. P., Rahmawati, & W, E. R. P. (2019). Deteksi dan Identifikasi Genera Bakteri Coliform Hasil Isolasi dari Minuman Lidah Buaya. *Jurnal Labora Medika*, *3*(1), 29–35.

Suhartomi., Gulo, K. N., Saragih, A. D., Martinus, A. R., & Ikhtiari, R. (2020). *Antioxidant Properties of Sweet Orange Peels in Several Fractions of Methanolic Extract*. *Ichimat 2019*, 371–378.

Sumartin, S. A., Lister, I. N. E., & Fachrial, E. (2024). Effectiveness Analysis of Antidiabetic Property from Dragon Fruit Peel Methanol Extract in Alloxan-Induced Diabetic Rats. *Biology, Medicine, & Natural Product Chemistry*, *13*(1), 57–62. https://doi.org/10.14421/biomedich.2024.131.57-62

Toruan, S. A. L., Manu, T. T., Evriarti, P. R., & Ikhsanita, Z. (2023). Pemanfaatan Air Kelapa Muda Sebagai Media Alternatif Mac Concey untuk Pertumbuhan Escherichia coli dan Salmonella typhi. *Journal of Indonesian Medical Laboratory and Science*, *4*(1), 25–36.

Utami, B. C., Yuliani, N. N. S., & Furtuna, D. K. (2021). Perbandingan Uji Aktivitas Antibakteri Filtrat Aquadest Umbi Bawang Suna (Allium schoenoprasum L.) Terhadap Pertumbuhan Streptococcus pneumoniae dan Escherichia coli Dengan Metode Difusi Cakram Kirby-Bauer. *Herb-Medicine Journal*, *4*(4), 51. https://doi.org/10.30595/hmj.v4i4.8812

Warpala, I. W. S., Widiyanti, N. L. P. M., Suryanti, I. A., & Wibawa, I. W. S. (2019). *Diversity Genera of Coliforms Bacteria in Buyan Lake*. *394*(Icirad 2019), 24–31. https://doi.org/10.2991/assehr.k.200115.005

Yanestria, S. M., Dameanti, F. N. A. E. P., Musayannah, B. G., Pratama, J. W. A., Witaningrum, A. M., Effendi, M. H., & Ugbo, E. N. (2022). Antibiotic resistance pattern of Extended-Spectrum β-Lactamase (ESBL) producing Escherichia coli isolated from broiler farm environment in Pasuruan district, Indonesia. *Biodiversitas*, *23*(9), 4460–4465.

Yanestria, S. M., Mudji, E. H., & Rahmaniar, R. P. (2020). *Antibakteri Nano Silver Terhadap Bakteri Escherichia coli Yang Diisolasi Dari Susu Sapi Mastitis*. *5*, 65–70.

Zebua, A. H. P., Nursyirwani, & Feliatra. (2020). Molecular Identification of Proteolitic Bacteria from Mangrove Sediment in Dumai Marine Station. *Asian Journal of Aquatic Sciences*, *3*(2), 179–188. https://doi.org/10.31258/ajoas.3.2.179-188