

Article

Application of *Citrus paradisi* Extract as a Natural Alternative for the Disinfection of Contaminated Surface Waters

Luis Cabanillas-Chirinos ^{1,*} , Moisés Gallozzo-Cárdenas ¹, Magaly De La Cruz-Noriega ¹ ,
Víctor Sánchez-Araujo ² and Pedro Palomino-Pastrana ²

¹ Centros e Institutos de Investigación, Universidad Cesar Vallejo, Trujillo 13001, Peru; mgallozzo@ucv.edu.pe (M.G.-C.); mdelacruz@ucv.edu.pe (M.D.L.C.-N.)

² Facultad de Ciencias de Ingeniería, Universidad Nacional de Huancavelica, Huancavelica 09001, Peru; victor.sanchez@unh.edu.pe (V.S.-A.); pedro.palomino@unh.edu.pe (P.P.-P.)

* Correspondence: lcabanillas@ucv.edu.pe

Abstract

Microbiological contamination of surface water represents a critical public health concern, while conventional disinfectants face limitations such as the generation of toxic by-products and the emergence of microbial resistance. In this study, the application of an ethanolic peel extract of *Citrus paradisi* (grapefruit), obtained by sonication at 40 kHz for 90 min at 55 °C using a 1:4 (*w/v*) solvent-to-solid ratio, was evaluated as a natural alternative for bacterial reduction in contaminated waters. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract were first determined against *Staphylococcus aureus* and *Escherichia coli*. The extract was then applied to samples of slightly contaminated surface water with bacterial loads between 10³–10⁴ CFU/mL and turbidity of 150 NTU, as well as to highly contaminated surface water with bacterial loads ≥10⁵ CFU/mL and turbidity of 250 NTU. Bacterial removal was assessed at 6, 12, and 24 h. FTIR and UV-Vis characterization of the extract confirmed the presence of flavonoids (naringin), terpenes (limonene), and phenolic compounds. Results showed MIC/MBC values of 2.5/5.0 mg/mL for *S. aureus* and 5.0/10.0 mg/mL for *E. coli*. In slightly contaminated water, the extract at 5.0 mg/mL achieved complete (100%) removal of both microorganisms after 12 h, whereas in highly contaminated water, removals ranged from 80–90% for *Staphylococcus* spp. and *E. coli*. Statistical analysis (ANOVA, Bonferroni) demonstrated significant differences (*p* < 0.05) between the extract and ethanol. These findings indicate that *Citrus paradisi* extract constitutes an effective, sustainable, and low-cost natural alternative for bacterial reduction in surface waters, contributing to the valorization of agro-industrial residues and to the achievement of Sustainable Development Goal (SDG) 6.

Keywords: *Citrus paradisi*; water disinfection; agro-industrial residues; antimicrobial activity; flavonoids; surface waters



Academic Editors:
Helvi Heinonen-Tanski and
Yuanyuan Zhang

Received: 22 May 2026

Revised: 1 July 2026

Accepted: 2 July 2026

Published: 7 July 2026

Copyright: © 2026 by the authors.
Licensee MDPI, Basel, Switzerland.
This article is an open access article
distributed under the terms and
conditions of the [Creative Commons
Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Microbiological contamination of water represents one of the greatest global public health challenges of the 21st century. According to the latest joint WHO and UNICEF report (2023), approximately 2.2 billion people lack access to safely managed drinking water, while 3.4 billion do not have adequate sanitation services [1–3]. This situation generates a devastating health burden: diarrheal diseases associated with the consumption of contaminated water cause approximately 485,000 deaths annually in vulnerable populations,

ranking as the second leading cause of child mortality in low-income countries [4,5]. In Latin America, it is estimated that 25% of surface water sources present fecal contamination above permissible limits, contributing to recurrent outbreaks of gastroenteritis, hepatitis A, and typhoid fever [6–8].

Conventional disinfection technologies such as chlorination, ozonation, and UV radiation present significant limitations. Chlorination, although effective and economical, generates disinfection by-products (DBPs) such as trihalomethanes and haloacetic acids, which are classified as harmful to health [9,10]. Furthermore, recent studies have documented the increase in microbial resistance due to misuse and overexposure to sublethal chlorine concentrations; strains of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* have shown increased resistance to imipenem [11,12]. The disadvantages of ozonation and UV systems include high energy costs, lack of residual effect, and reduced efficacy in turbid waters [13,14]. These limitations have driven the search for alternative methods that are accessible, sustainable, and of low toxicological risk, aligned with the principles of “green chemistry” and the circular economy.

Agro-industrial residues represent between 15–20 million tons annually worldwide, being largely underutilized and generating environmental problems due to inadequate disposal [15]. Industrial citrus processing produces large volumes of residues, including peels, membranes, seeds, and pulp derived from juice extraction [16]. These by-products, traditionally considered waste, possess high valorization potential due to their content of pectin, polyphenols, carotenoids, and essential oils. Such compounds can be transformed into valuable inputs for the food, pharmaceutical, and cosmetic industries [16,17].

Emerging research has shown that *Citrus paradisi* extracts possess remarkable antimicrobial capacity, whose effectiveness varies significantly depending on the type of extract, the plant part used, and the bacterial strain tested [18,19]. The essential oil from the peel exhibits bactericidal effects at very low concentrations, between 0.192 and 0.768 mg/mL, against pathogens such as *K. pneumoniae*, *Acinetobacter baumannii*, *E. coli*, and *P. aeruginosa* [20]. Regarding peel extracts, the choice of solvent directly influences their potency [19,21]. Ethanolic or hydroalcoholic peel extracts are generally more effective than aqueous ones, showing bactericidal concentrations ranging from 6.25 to 25 mg/mL against *S. aureus* ATCC 6538 and clinical strains of *S. aureus*, *E. coli*, and *Enterococcus faecalis* [21]. Other studies have reported that naringin-rich extracts can inhibit *E. coli* at 2.5 mg/mL and *S. aureus* at 5 mg/mL [22].

Recent phytochemical studies have characterized the bioactive profile of *Citrus paradisi*, linking its antimicrobial activity to a rich composition of bioactive compounds such as flavonoids, terpenes, and furanocoumarins present in the peel, seeds, and juice. Among flavonoids, naringin is predominant in the peel and metabolizes into naringenin, acting as a potent antimicrobial agent with proven efficacy against *E. coli* and *S. aureus* [22,23]. Within terpenes, D-limonene is the major compound in the essential oil and is reported to be responsible for bactericidal effects by causing damage to cell membranes [20,24]. Other compounds such as furanocoumarins and phenolic acids, mainly obtained through organic solvent extraction, have also demonstrated bactericidal activity in extracts [23–26].

Despite this potential, knowledge gaps persist, as most studies have focused on pharmaceutical or food applications, with limited evidence regarding the disinfectant efficacy of extracts in complex aqueous matrices with high organic loads [27]. This research addresses those gaps by introducing several distinctive elements. Unlike earlier investigations that primarily reported MIC and MBC values for pathogens under controlled laboratory conditions [18–26], our study evaluates the effectiveness of the extract in real surface and wastewater samples, which contain complex organic and inorganic compounds that may interfere with antimicrobial activity. Furthermore, it adopts a rarely explored kinetic

perspective, assessing bacterial reduction at 6, 12, and 24 h. Another notable distinction is that, while previous studies have focused mainly on pharmaceutical or food applications [16,17,27], our work specifically examines water antimicrobial activity, a critical public health application with unique requirements and challenges. These considerations emphasize the importance of studies such as ours, which not only validate the antimicrobial efficacy of *Citrus paradisi* extract but also contribute to advancing knowledge that supports the achievement of Sustainable Development Goal (SDG) 6, ensuring access to safe and high-quality water for communities in need.

2. Materials and Methods

2.1. Collection and Preparation of Plant Material

Citrus paradisi peel residues were collected from local markets in Trujillo, where this fruit is commonly sold. The material was transported to the laboratory in single-use bags. As a pretreatment, the residues were washed with running water to remove excess dirt and adhered organic matter. The clean material was then oven-dried at 40 °C for 3–4 days to reduce moisture prior to milling. The dried product was pulverized and sieved through a No. 60 mesh (ASTM E-11) to obtain a homogeneous powder, which was packaged in opaque single-use containers and stored at room temperature [28,29].

2.2. Extract Preparation

Sonication was employed as a clean extraction method. The process used a 1:4 (*w/v*) ratio between the solid (50 g of plant powder) and the solvent (200 mL of 99% ethanol). Extraction conditions were set at 55 °C for 90 min under constant sonication at 40 kHz. The resulting extract was clarified by filtration through Whatman No. 1 paper and concentrated using a rotary evaporator (ISOLAB). Finally, it was sterilized by vacuum filtration with 0.22 µm membranes and stored in sterile amber vials [30,31]. This product was designated as the mother concentrated extract, and its concentration was determined using the pycnometer method.

The extraction yield was determined gravimetrically by weighing the dry extract obtained after rotary evaporation and comparing it to the initial dry weight of the plant material. The extraction yield was calculated as Extraction Yield (%) = (Weight of extract obtained/Weight of dry plant material) × 100, yielding a value of $18.5 \pm 1.2\%$ (*w/w*) based on the dry weight of the peel. This yield is consistent with values reported in the literature for ultrasound-assisted extraction of citrus peels, which generally range between 10% and 25%, depending on the solvent type, extraction conditions, and citrus variety [28,30].

2.3. Evaluation of Antimicrobial Potential

2.3.1. Bacterial Sensitivity to the Extract

Certified ATCC strains (American Type Culture Collection) were used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract against *S. aureus* (ATCC 12493) and *E. coli* (ATCC 33876). The broth macrodilution method was applied following CLSI-M07 guidelines, using Mueller Hinton (MH) broth. An inoculum equivalent to 0.5 McFarland scale (approximately $1\text{--}2 \times 10^8$ CFU/mL) was prepared, diluted, and added to broth tubes containing the extract to reach a final concentration of 5×10^5 CFU/mL. The initial working concentration for these assays was 40 mg/mL. Each evaluation was performed in triplicate, and the results were used to establish the extract concentrations applied in subsequent experimental trials [21,32].

2.3.2. Experimental Trials for Antimicrobial Activity of Contaminated Water

Trials were conducted on two different samples of naturally contaminated water. First, river water (slightly contaminated) with microbial loads between 10^3 – 10^4 CFU/mL and turbidity of 150 NTU was used. Second, wastewater (highly contaminated) with microbial loads $>10^4$ CFU/mL and turbidity of 250 NTU was tested.

The samples were collected directly from natural sources. River water was obtained from the Mochica canal, Trujillo, Peru, at coordinates of $8^{\circ}05'27''$ S, $79^{\circ}02'58''$ O, while wastewater was collected from a canal contaminated with municipal sewage near its mouth at Buenos Aires Norte beach, Trujillo, Peru, at coordinates of $8^{\circ}07'54''$ S, $79^{\circ}04'03''$ O. In both cases, the water bodies are influenced by agricultural runoff and urban discharges, whereas the sewage canal receives domestic wastewater with inadequate treatment from the Trujillo municipal wastewater treatment plant. They were immediately transported to the laboratory for experimental assays. Treatments included 99% ethanol and two different concentrations of the ethanolic extract, with sterile distilled water as control. Experiments were conducted in 100 mL test tubes, where 90% corresponded to the water sample and 10% to the added solution (sterile distilled water, 99% ethanol, 2.5 mg/mL extract, or 5.0 mg/mL extract).

The final concentration of ethanol in the extract-treated samples was calculated as follows: the extract was prepared with 99% ethanol and applied at a 10% (*v/v*) ratio to the water samples, resulting in a residual ethanol concentration of 9.9% (*v/v*) in the test solutions.

Bactericidal effects were evaluated through microbiological counts at 0, 6, 12, and 24 h (time 0 corresponded to the analysis of untreated water samples). Microbiological counts focused on quantifying pathogenic bacteria: pathogenic staphylococci on Mannitol Salt Agar (MSA) and coliforms on Eosin Methylene Blue Agar [33,34]. Each procedure was repeated five times per water matrix to obtain statistically relevant data.

Bacterial reduction was calculated using the percentage of removal, determined as $[(\text{initial CFU/mL} - \text{final CFU/mL}) / \text{initial CFU/mL}] \times 100$, to facilitate comparison with studies that report results in this format. The values of logarithmic reduction, calculated as $\log_{10} (\text{initial CFU/mL} / \text{final CFU/mL})$, were considered to compare the results obtained with the existing literature.

2.4. Spectrophotometric Characterization of the Extract

Spectrophotometric characterization of the extract was performed using two techniques. The first evaluation employed a UV-Vis spectrophotometer (Thermo Fisher Scientific, Madison, WI, USA), and the second characterization used an FT-IR iS50 spectrometer (Thermo Fisher Scientific, Madison, WI, USA). UV-Vis analysis allows the identification of chromophores and conjugated systems, such as those found in flavonoids and phenolic compounds, which typically display characteristic absorption bands in the 270–350 nm region [35–37]. FTIR analysis allows the identification of characteristic functional groups (e.g., O–H, C–H, C=O, C–O–C) based on their specific absorption bands in the range of 4000 – 400 cm^{-1} [36,38].

2.5. Statistical Analysis

All experimental data were recorded in Microsoft Excel spreadsheets to calculate percentage and logarithmic reductions in bacteria in each treatment, and graphs were generated using OriginPro 9.0 software. Statistical analyses to determine significant differences in antimicrobial activity among treatments were performed using SPSS version 26. Data normality was assessed with the Shapiro–Wilk test, and inferential statistics were conducted using ANOVA followed by Post Hoc tests.

3. Results and Discussion

3.1. Antimicrobial Activity of the Extracts

Table 1 presents the antimicrobial activity of the *Citrus paradisi* extract, showing greater efficacy against *S. aureus* (ATCC 12493) than against *E. coli* (ATCC 33876). The MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values confirm the antimicrobial activity of the extract and reveal differences between the microorganisms tested. MIC and MBC values for *S. aureus* were 2.50 and 5.00 mg/mL, respectively, whereas MIC and MBC values for *E. coli* were 5.00 and 10.0 mg/mL. These results indicate that the concentration required to inhibit and eliminate *E. coli* is approximately twice that needed for *S. aureus*, suggesting greater intrinsic resistance of the Gram-negative bacterium to the extract.

Table 1. Antimicrobial activity of *Citrus paradisi* extract.

Microorganism	MIC ¹	MBC ²
<i>Staphylococcus aureus</i> ATCC 12493	2.5 mg/mL	5.0 mg/mL
<i>Escherichia coli</i> ATCC 33876	5.0 mg/mL	10.0 mg/mL

Note: ¹ Minimum Inhibitory Concentration (MIC); ² Minimum Bactericidal Concentration (MBC).

The results in Table 1 are consistent with those reported by Arsène et al. (2021) [21], who found that aqueous and ethanolic extracts of *Citrus paradisi* peel exerted stronger effects against Gram-positive bacteria compared to Gram-negative ones. In that study, MIC values for *S. aureus* (1.56–3.12 mg/mL) were notably lower than those for *E. coli* (12.5 mg/mL), reinforcing the hypothesis of greater sensitivity of Gram-positive pathogens to the extract [21]. This disparity in bacterial sensitivity is often attributed to structural differences in the cell wall: Gram-positive bacteria such as *S. aureus* possess a thick peptidoglycan layer that does not act as an effective permeability barrier against hydrophilic agents, whereas Gram-negative bacteria such as *E. coli* have an outer membrane composed of phospholipids and lipopolysaccharides that confers intrinsic resistance by functioning as a selective barrier against polar molecules [21,24].

However, specialized literature reveals divergent results. Nguyen et al. (2024) reported opposite findings, with lower MIC values for *E. coli* (2.5 mg/mL) than for *S. aureus* (5 mg/mL) when using ultrasound-assisted extraction, suggesting that extraction methodology can modify the antimicrobial activity profile [22]. Other studies highlight the influence of solvents, reporting that ethanolic or alcoholic extracts are more effective than aqueous ones [19,26]. Likewise, the bactericidal concentrations in our study were considerably higher compared to those obtained with essential oils, where bactericidal concentrations range between 0.384–0.768 mg/mL [20]. This suggests that volatile components such as limonene (which constitutes >85% of the oil) possess much stronger antimicrobial activity than compounds present in crude extracts or decoctions. While the study of water disinfection aligns with the general premise of greater effectiveness against Gram-positive bacteria, the available evidence shows that the minimum bactericidal concentration is strongly influenced by methodological factors, such as the type of extract, the solvent used, and the extraction technique.

Experimental results in Figure 1 demonstrate that *Citrus paradisi* extract exerts significant, concentration-dependent antimicrobial activity against *Staphylococcus* spp. and *E. coli* in contaminated water. For *Staphylococcus* spp. in slightly contaminated water (Figure 1a), both 2.5 mg/mL and 5.0 mg/mL concentrations produced notable reductions in bacterial counts, exceeding the effect of ethanol alone. These trends were accentuated in highly contaminated water (Figure 1c), where 5.0 mg/mL showed the greatest efficacy, suggesting stronger inhibitory action even under high microbial loads. For *E. coli*, results were more

differentiated. In slightly contaminated water (Figure 1b), 5.0 mg/mL achieved a considerable reduction, though less pronounced than that in *Staphylococcus* spp., while 2.5 mg/mL showed moderate effects. Under highly contaminated conditions (Figure 1d), only the higher concentration (5.0 mg/mL) maintained relevant antimicrobial activity, indicating that *E. coli* (Gram-negative) exhibits greater resistance compared to *Staphylococcus* spp. (Gram-positive).

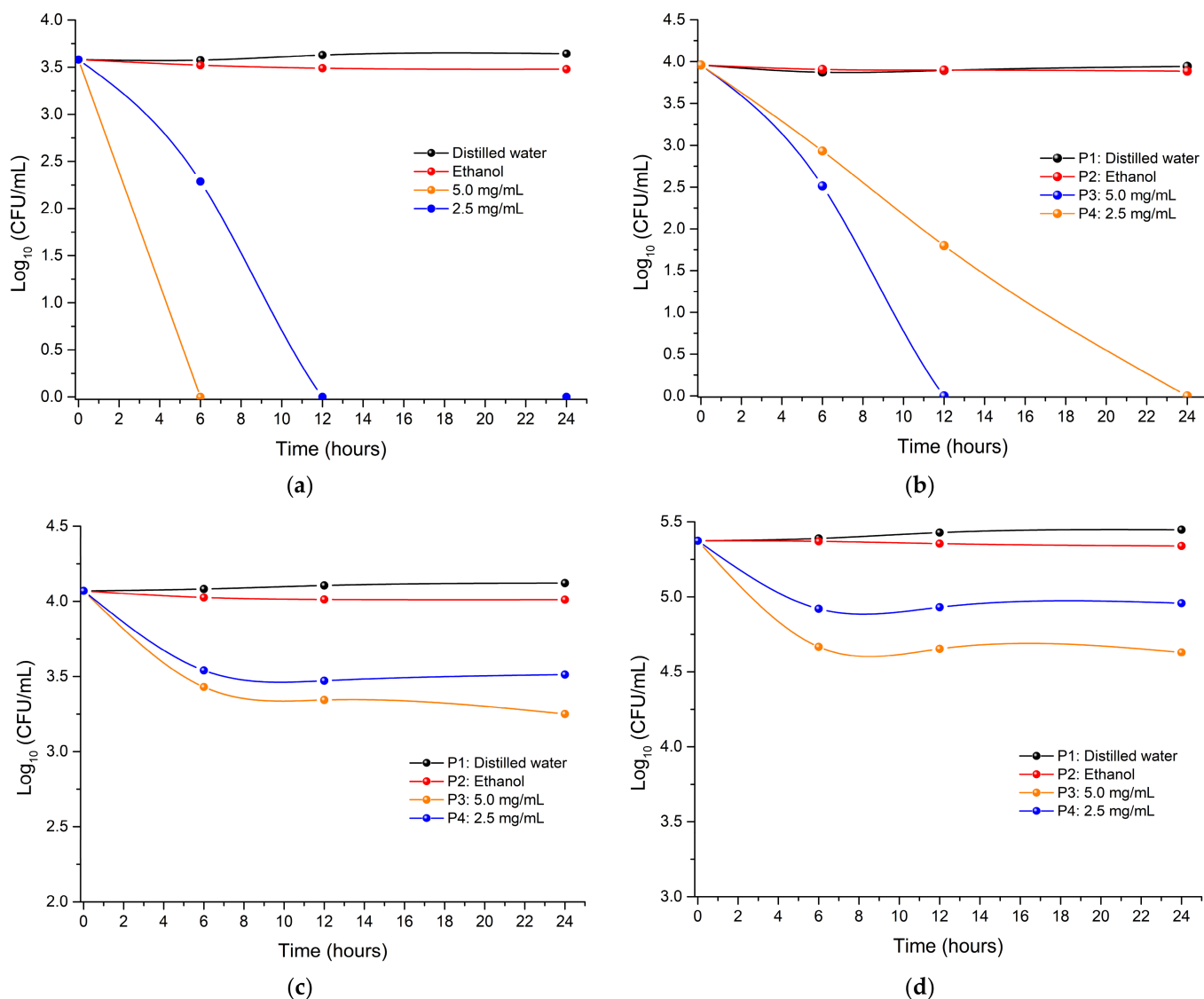


Figure 1. Temporal evolution of *Staphylococcus* spp. (a,c) and *E. coli* (b,d) counts in slightly contaminated water (a,b) and highly contaminated water (c,d) after treatment with distilled water (control), ethanol, and *C. paradisi* extract at 2.5 and 5.0 mg/mL. Data represent mean values ($n = 5$).

Results in Figure 2, expressed as removal percentage reductions in viable bacterial counts (CFU/mL), confirm and quantify the differential antibacterial activity of treatments. Complete removal (100%) indicates bactericidal activity, as no viable cells were detected on the plates. In slightly contaminated water (Figure 2a), *Citrus paradisi* extract at 5.0 mg/mL showed the highest removal percentages for both microorganisms, being especially effective against *Staphylococcus* spp., with values close to or exceeding 90%. The 2.5 mg/mL concentration also produced significant removal, though lower, while ethanol exhibited moderate and consistently inferior effects compared to both extract concentrations. For *E. coli* under the same condition, percentages were notably lower, again highlighting its

greater resistance. In highly contaminated water (Figure 2b), the concentration-dependent effectiveness persisted, although overall removal percentages were lower due to the higher initial microbial load. The 5.0 mg/mL extract remained the most effective treatment, achieving the greatest reductions, particularly against *Staphylococcus* spp., while ethanol showed limited efficacy. It is also observed that there is a significant difference between the *C. paradisi* extract at 2.5 and 5.0 mg/mL and the 99% ethanol control, considering that the residual ethanol concentration in the extract-treated samples was only 9.9% (v/v). This indicates that the bactericidal effect is primarily attributable to the bioactive compounds present in the extract rather than to the solvent.

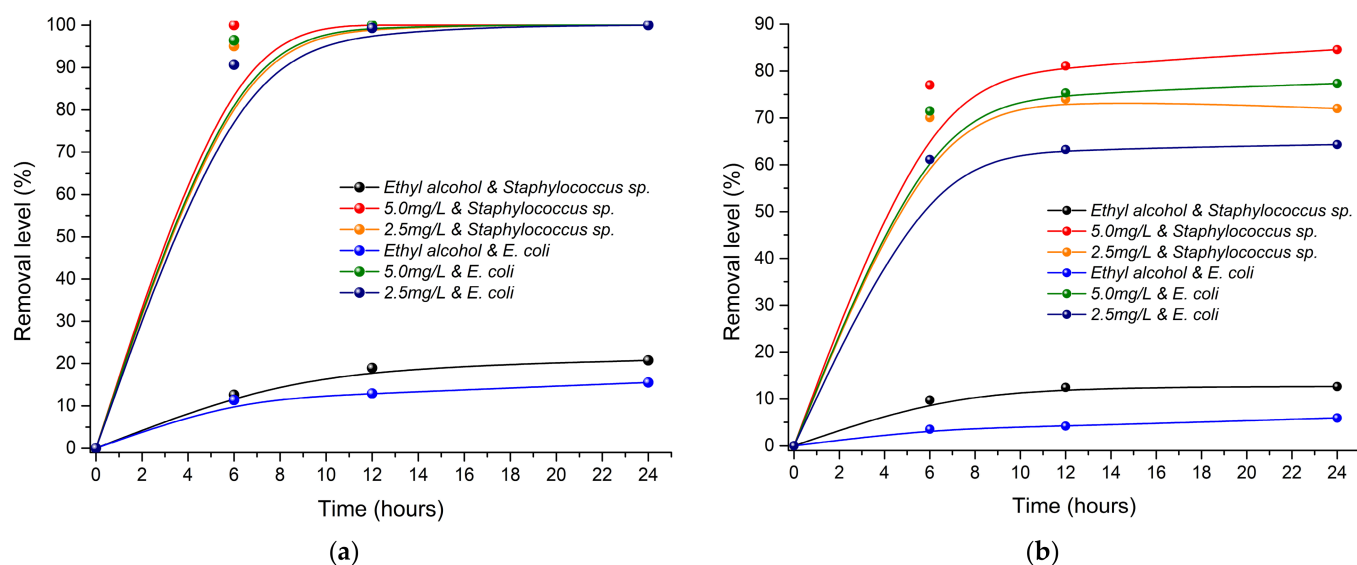


Figure 2. Percentage reduction in viable bacterial counts (CFU/mL) of *Staphylococcus* spp. and *E. coli* at 6, 12, and 24 h of treatment in: (a) slightly contaminated water and (b) highly contaminated water. Complete reduction (100%) indicates bactericidal activity (no viable cells detected). Data represent mean values ($n = 5$).

A review of scientific literature reveals few studies reporting bacterial reduction in logarithmic units or percentage removal in liquid media using *Citrus* spp. extracts. Most phytochemical and microbiological studies typically report MIC, MBC, and inhibition zone diameters (mm) [18–26]. This distinction is crucial. While MIC/MBC values provide a useful baseline, they do not account for the interfering effects of a complex water matrix. Our study provides novel, quantitative data on the kinetic removal of bacteria in real water samples, demonstrating that the *C. paradisi* extract retains significant activity even in the presence of natural organic matter and competing microflora. This positions the extract as a practically viable candidate for point-of-use water treatment, particularly in settings where the tested turbidity (150–250 NTU) and contamination levels (10^3 – 10^5 CFU/mL) are common, such as in many rural communities of Latin America and other developing regions. However, some studies quantify antimicrobial activity of surface disinfectants formulated with citrus extracts against pathogens, allowing comparison with removal levels observed in this study. The product Biosecur® (Quebec, QC, Canada), formulated with citrus extracts and used as a surface disinfectant, reduced *Vibrio vulnificus* by 3–6 log units when applied at concentrations between 0.5–2.0% [39]. Similarly, Citrox® Kimbolton, Reino Unido (citrus extract) applied to fresh foods significantly reduced survival of *E. coli* O157:H7 and *Listeria monocytogenes*, achieving reductions of 2.8–4.8 log CFU/g for *E. coli* O157:H7 in treated samples [38]. These findings are comparable to our experimental results, where reductions of up to 4 log units were observed in slightly contaminated water within

24 h, while in highly contaminated water, maximum removal reached 1 log unit within the same period.

It is important to clarify that the reference to commercial products such as Biosecur[®] and CitroX[®] in this discussion serves solely to benchmark the bactericidal efficacy observed in our study against reported values for other citrus-based formulations. However, our approach differs fundamentally from these commercial products in scope and application. Biosecur[®] is marketed primarily as a surface disinfectant [37], and CitroX[®] is used as a preservative for fresh foods [36]. In contrast, our study specifically validates the application of a *Citrus paradisi* peel extract for the antimicrobial activity of surface water, a critical public health need often unaddressed by these commercial products. Furthermore, our methodology valorizes an agro-industrial waste (peel) through a simple, low-cost sonication process, making it a potentially decentralized and locally adaptable technology, particularly relevant for resource-limited communities. This aligns with circular economy principles and represents a distinct contribution to the field of sustainable water treatment.

Regarding removal percentages, literature shows that natural coagulants such as baker's yeast can eliminate 95–99% of coliforms in water samples with turbidity up to 500 NTU [35]. A similar study using *Plantago ovata* seed extract achieved near-total removal of total coliforms and streptococci in river water with low bacterial contamination (600 CFU/100 mL) [36]. These values are considerably lower than those obtained in our study, where experiments with slightly contaminated water (4×10^3 – 1×10^4 CFU/mL) achieved 90–100% removal of *E. coli* and *S. aureus*. In contrast, experiments with highly contaminated water (1×10^4 – 2×10^5 CFU/mL) achieved 60–85% removal of *E. coli* and *S. aureus*.

An important observation in our results is the slight decrease in the removal of *E. coli* with the ethanol control in slightly contaminated water between 12 and 24 h (from 25.45% to 17.86%). This pattern, which was not observed in highly contaminated water, can be explained by several factors. First, bacterial regrowth is a possibility, since ethanol at low concentrations tends to act more as a bacteriostatic than a bactericidal agent, allowing some cells to survive and resume growth. Moreover, *E. coli*, as a Gram-negative bacterium, possesses an outer membrane that confers relative resistance to hydrophilic agents, which could facilitate metabolic adaptation to low ethanol concentrations [21,24]. In addition, experimental variability and minor counting errors in the plate count method may have contributed to this fluctuation; however, the normality test (Shapiro–Wilk, $p > 0.05$) indicates that variability remained within acceptable limits. Taken together, these elements acknowledge the reviewer's observation and provide a coherent explanation for the unexpected behavior.

3.2. Statistical Analysis of the Data

The data were analyzed using a one-way analysis of variance (ANOVA) followed by Bonferroni post hoc tests. One-way ANOVA was selected because the primary objective was to compare bacterial reduction percentages among independent treatment groups (extract at 2.5 mg/mL, extract at 5.0 mg/mL, and ethanol control) at each individual time point (6, 12, and 24 h). Each time point was analyzed independently, as the experimental replicates ($n = 5$ per treatment and per time point) were independent. The Shapiro–Wilk test confirmed data normality ($p > 0.05$), validating the use of parametric tests. Bonferroni correction was applied to control the overall error rate in multiple comparisons. While a repeated-measures ANOVA or a mixed-effects model would be more appropriate for analyzing temporal trends and time-by-treatment interactions, the current approach was considered sufficient for the exploratory nature of the study, where the primary objective was to identify significant differences among treatments at each time point.

To verify compliance with the assumptions required for the subsequent application of parametric statistical tests, the normality of the data distribution was assessed using the Shapiro–Wilk test. The results revealed that probability values (p -values) were higher than the established significance level of 0.05 across all experimental groups analyzed (Appendix A.1). Consequently, the null hypothesis was not rejected, allowing the assumption that the data followed a normal distribution. This statistical finding indicates that bacterial removal percentages recorded at 6, 12, and 24 h for the different treatments (ethanol and *Citrus paradisi* extract at 2.5 mg/mL and 5.0 mg/mL) in contaminated water samples were consistent with normality. This conclusion was qualitatively corroborated in Figure 3 through visual inspection of scatter plots for each type of contaminated water sample. In Figure 3a,b, a symmetrical distribution of points around the median of each group is observed, without evidence of anomalous clustering at extreme ranges. Likewise, no outliers with substantial deviation from the main data cluster were identified.

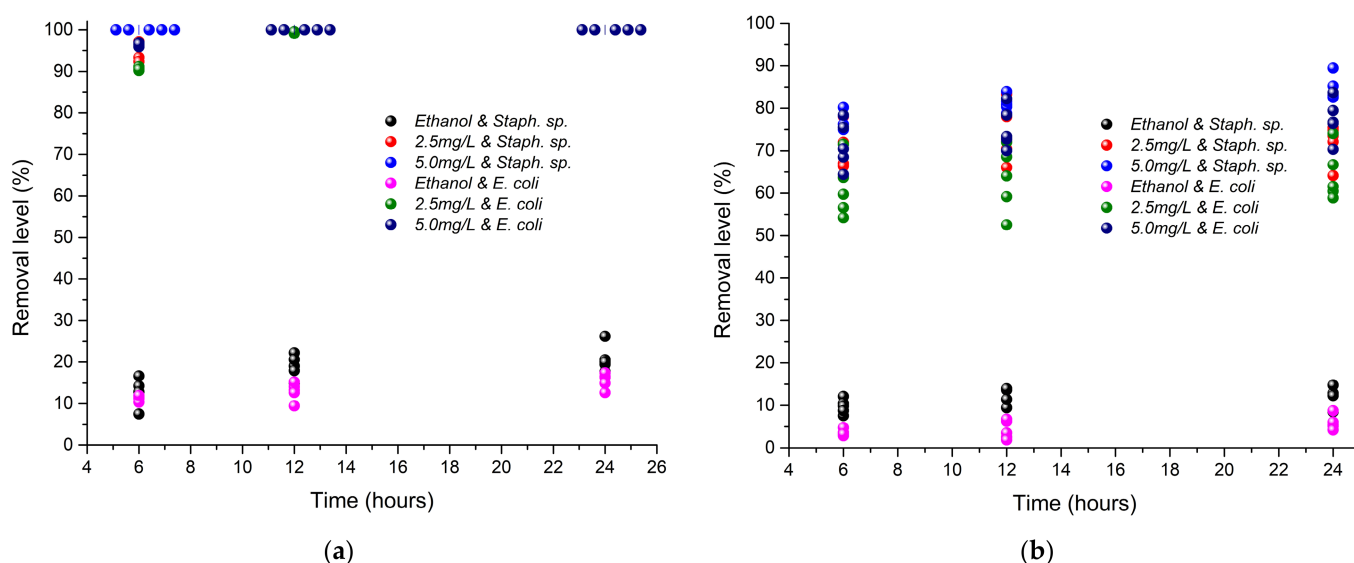


Figure 3. Scatter plot representation of bacterial removal percentages in contaminated waters: (a) Scatter plot of *Staphylococcus* spp. and *E. coli* removal percentages in slightly contaminated water; (b) Scatter plot of *Staphylococcus* spp. and *E. coli* removal percentages in highly contaminated water.

To determine whether statistically significant differences existed among the means of three or more independent groups, ANOVA (Analysis of Variance) was performed. The resulting p -value was below 0.05, indicating significant differences among the groups compared. Based on this finding, Bonferroni post hoc tests were conducted.

Evaluation of box-and-whisker plots (Box-Plots) corresponding to bacterial removal percentages of *Staphylococcus* spp. and *E. coli* in slightly contaminated water, complemented by Bonferroni post hoc results in Appendix A.2, showed that at 6 h (T6H), statistically significant differences ($p < 0.05$) were identified in most comparisons between ethanol- and extract-treated groups for both *Staphylococcus* spp. and *E. coli*. This distinction is visually evident in Figure 4a, where clear separation between medians and minimal overlap of interquartile ranges is observed. However, direct comparison between the two extract concentrations (2.5 mg/mL vs. 5.0 mg/mL) in *Staphylococcus* spp. did not reach statistical significance ($p = 0.077$), reflected graphically in the notable overlap of their box-and-whisker plots. In contrast, for *E. coli*, the difference between these concentrations was significant ($p = 0.022$), consistent with reduced overlap in their distributions. At 12 h (T12H) and 24 h (T24H), Bonferroni results indicated a different scenario: nearly all comparisons among extract treatments (regardless of concentration or bacterial strain) yielded non-significant p -values ($p = 1.000$). This statistical uniformity corresponded with the homogeneity observed

in box plots, where medians aligned closely and boxes overlapped extensively. Nevertheless, differences between ethanol and extract groups remained statistically significant ($p < 0.05$) at both time points, consistent with the visual separation still evident in the plots.

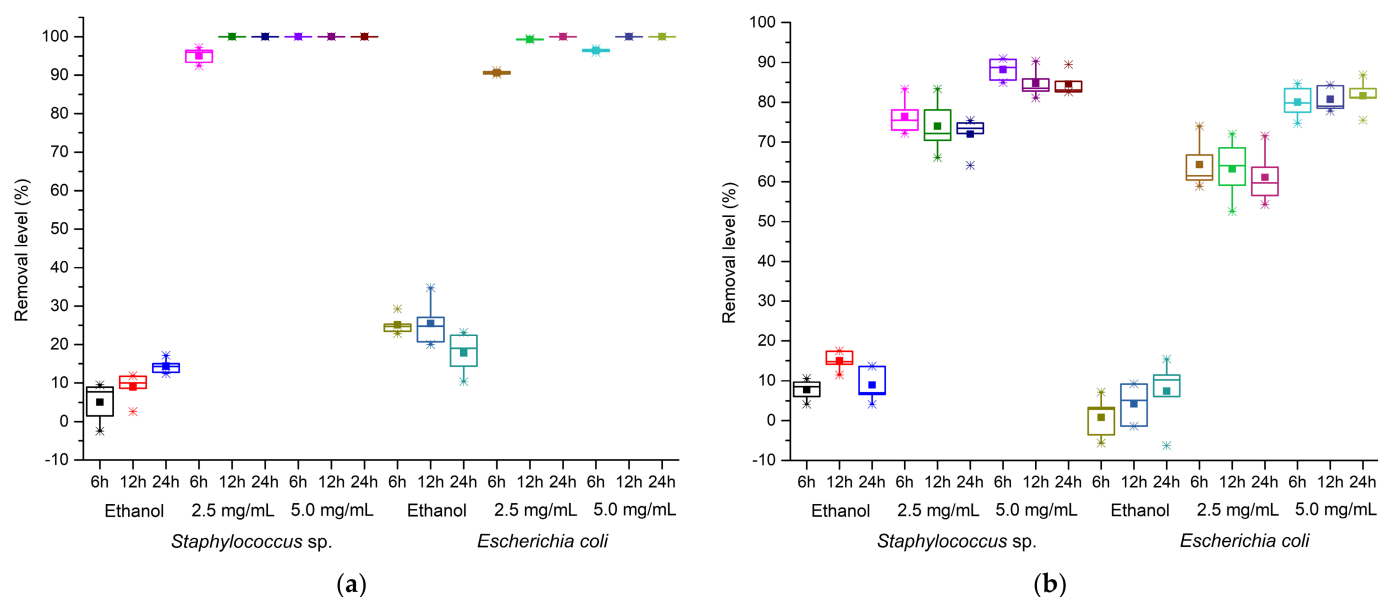


Figure 4. Group comparisons using Box-Plot graphs: (a) Box-Plot of *Staphylococcus* spp. and *E. coli* removal percentages in slightly contaminated water; (b) Box-Plot of *Staphylococcus* spp. and *E. coli* removal percentages in highly contaminated water.

Evaluation of box-and-whisker plots for bacterial removal percentages in highly contaminated water, complemented by Bonferroni post hoc results in Appendix A.3, showed that at 6 h (T6H), highly significant differences ($p < 0.01$) were identified between most extract treatments and ethanol for both bacterial strains. This statistical distinction was visually reflected in Figure 4b by clear vertical separation between group boxes, with minimal overlap of interquartile ranges. Specifically, a significant difference ($p = 0.005$) was observed between the two extract concentrations (2.5 mg/mL vs. 5.0 mg/mL) for *Staphylococcus* spp., suggesting a higher median and overall elevated position of the 5.0 mg/mL box. For *E. coli*, this comparison was also significant ($p = 0.004$), indicating an initial dose-dependent response represented by a stepped arrangement of boxes. At 12 h (T12H), extract treatments maintained significant superiority over ethanol ($p < 0.001$). However, direct comparison between the two concentrations for *Staphylococcus* spp. was at the threshold of statistical significance ($p = 0.051$), suggesting greater overlap and closer medians than at T6H. For *E. coli*, the difference between 2.5 mg/mL and 5.0 mg/mL was not significant ($p = 0.051$), though significant differences were detected between 5.0 mg/mL and ethanol-treated *Staphylococcus* spp., reflecting complex interactions between bacterial species and treatments. At 24 h (T24H), the pattern of significant extract effectiveness over ethanol persisted. Notably, for *Staphylococcus* spp., the difference between concentrations regained statistical significance ($p = 0.021$), interpreted in the box plot as a reopening or maintenance of the visual gap between 2.5 mg/mL and 5.0 mg/mL. For *E. coli*, the comparison between concentrations was not significant ($p = 0.071$), but significant differences were observed between the lower concentration of *E. coli* (2.5 mg/mL) and the higher concentration of *Staphylococcus* spp. (5.0 mg/mL), highlighting possible specificity of the effect.

In summary, there is consistent correspondence between data distributions inferred from box plots and quantitative results of multiple comparisons. This coherence underscores that under highly contaminated conditions, *Citrus paradisi* extract exhibits signifi-

cantly greater bactericidal effectiveness than ethanol from the earliest hours, with graphical visualization confirming these statistical relationships by showing clear separations where significant differences exist and overlaps where Bonferroni tests did not detect them.

3.3. Spectrophotometric Characterization of the Extracts

Spectrophotometric characterization of the *Citrus paradisi* extract is shown in Figure 5, revealing the presence of functional groups and bioactive compounds consistent with its previously observed antimicrobial activity.

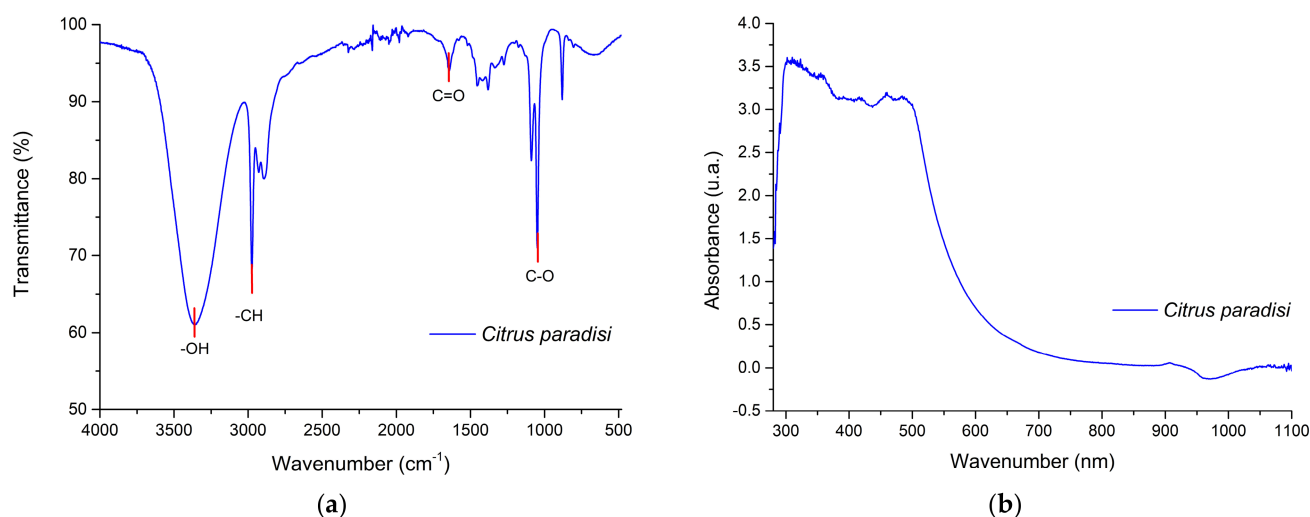


Figure 5. Spectrophotometric characterization of *Citrus paradisi* extract: (a) FTIR spectra and (b) UV-Vis spectrum (200–800 nm).

In the FTIR spectrum (Figure 5a), characteristic peaks were identified that allow assignment of the most relevant functional groups [37]. A broad and strong band around 3200–3600 cm^{-1} indicates O–H stretching, compatible with phenols, alcohols, and free carboxylic acids. Signals at 2920–2850 cm^{-1} correspond to aliphatic C–H stretching of methyl and methylene groups, associated with terpenes and lipids. A pronounced band at 1740–1700 cm^{-1} suggests the presence of C = O groups (esters or carbonyls of organic acids), while absorptions at 1650–1600 cm^{-1} are assigned to aromatic C=C or conjugated carbonyls, typical of flavonoids and phenolic acids. Regions between 1310–1000 cm^{-1} show intense C–O and C–O–C vibrations, indicative of ethers, esters, and glycosides (flavonoid glycosylations). In the fingerprint region (900–600 cm^{-1}), aromatic C–H bands and out-of-plane deformations reinforce the presence of aromatic compounds and ring substitutions. Overall, the pattern suggests a mixture rich in phenols/flavonoids, esters, and terpenoid components, consistent with expected phytochemical profiles in citrus [40,41].

The UV-Vis spectrum (Figure 5b) complements this analysis, showing a strong and broad absorption band between 270–350 nm. The absorption maximum around 280–290 nm is characteristic of flavonoids, specifically flavanones such as naringin, while the shoulder or less intense peak in the 320–350 nm region may be associated with flavonols or extended conjugation systems [42,43]. The absence of significant absorption above 400 nm confirms the lack of pigments such as chlorophylls or carotenoids in high concentration, indicating selective extraction of phenolic compounds [44]. Together, these spectral profiles confirm that the extract is rich in polyphenolic secondary metabolites, particularly flavonoids, widely recognized for their antioxidant and antimicrobial properties, thus providing a chemical basis for the inhibitory effects observed against *Staphylococcus* spp. and *E. coli*.

FTIR characterization of the *Citrus paradisi* extract revealed key functional groups that can be hypothetically related to antimicrobial activity (Table 2). The broad band in the

3200–3600 cm^{-1} region confirms the presence of O–H groups associated with phenols, phenolic acids, and flavonoids such as quercetin and hesperidin [37,40,41,45,46]. These compounds may act through destabilization of bacterial membranes, chelation of essential metal ions, induction of oxidative stress, enzyme inhibition, and biofilm disruption [45–47]. The signal at 2920–2850 cm^{-1} corresponds to aliphatic C–H bonds, characteristic of terpenes such as limonene, which contribute to lipid bilayer disorganization, increased cell permeability, and loss of intracellular content [45,48,49]. Peaks in the 1740–1700 cm^{-1} and 1685–1630 cm^{-1} regions are assigned to carbonyl (C=O) groups and aromatic or conjugated rings (C=C), present in lactones, anthraquinones, and flavonoids such as naringenin, which can interact with bacterial proteins and enzymes, altering their function [21,50,51]. Finally, bands between 1310–1000 cm^{-1} indicate the presence of C–O and C–O–C bonds typical of glycosides (e.g., hesperidin) [37,40,41,50], which enhance solubility and bioavailability of active compounds, facilitating their interaction with bacterial cell walls [52]. The correlations between functional groups and proposed mechanisms in Table 2 constitute working hypotheses grounded in the literature, which should be experimentally validated in future studies through mechanistic assays and bio-guided fractionation.

Table 2. Correlation between FTIR bands, identified functional groups, and proposed antimicrobial mechanisms for *Citrus paradisi* extract.

Range (cm^{-1})	Functional Group	Spectral Assignment	Probable Compounds	Proposed Antimicrobial Mechanism
0–3600	O–H (phenols, phenolic acids)	Broad stretching of alcohols/phenols/carboxylic acids	Flavonoids (quercetin, hesperidin); phenolic acids (caffeic acid)	Membrane disruption; ion chelation; oxidative stress induction; enzyme inhibition; biofilm adhesion blocking [45–47]
2920–2850	Aliphatic C–H (terpenes/essential oils)	Asymmetric/symmetric CH_2/CH_3 stretching	Monoterpenes (limonene, linalool, geraniol); fatty acids	Lipid bilayer disorganization; increased permeability; ATP loss; rapid bactericidal effect [45,48,49]
1740–1700; 1685–1630	C=O carbonyl; aromatic C=C; conjugated C=O	C=O stretching of esters and lactones; aromatic ring vibrations	Monoterpenes (limonene); fatty acids; lactones; anthraquinones; quercetin; rutin; naringenin	Protein/enzyme interaction; permeability alteration; bactericidal activity via protein target modification [21,49–51]
1310–1000; 1200–1140	C–O/C–O–C (glycosides, ethers)	Stretching of ethers, esters, and glycosidic bonds	Flavonoid glycosides (hesperidin); glucosides; phenolic esters	Improved solubility and transport; glycosides modulate bioavailability and release active aglycones; interaction with bacterial cell wall [52]

It is important to acknowledge the limitations of this study, which should be addressed in future research. The main limitation lies in the absence of chromatographic techniques (HPLC-MS) that would allow specific identification and quantification of the compounds responsible for the observed activity, thereby facilitating more precise correlations between chemical composition and antimicrobial potency. Therefore, the correlations established between the functional groups detected by FTIR and UV-Vis and the proposed mechanisms of action should be interpreted as working hypotheses rather than definitive conclusions. Moreover, while we provided data on turbidity and bacterial load, we ac-

knowledge that other physicochemical parameters, such as electrical conductivity, pH and dissolved organic matter, were not considered in the analysis of this study. These factors may influence antimicrobial efficacy and should be included in future investigations to better understand the performance of the extract under diverse water quality conditions. Nonetheless, the comparative experimental design employed allowed us to conduct a valid evaluation of antimicrobial activity, and the statistically significant differences ($p < 0.05$) observed among treatments confirm that the bactericidal effect is robust and primarily attributable to the bioactive compounds present in the extract, regardless of the unmeasured physicochemical parameters.

We recognize that natural surface waters harbor diverse microbial communities, including viruses, protozoa, fungi, and a wide range of bacterial species beyond the two indicator organisms evaluated in this study (*S. aureus* and *E. coli*). However, these organisms were selected for several reasons: (i) *E. coli* is the primary indicator of fecal contamination and is widely used in water quality assessment worldwide [2,3]; (ii) *S. aureus* serves as an indicator of pathogenic staphylococci, including antibiotic-resistant strains that represent a significant public health concern [11,12]; and (iii) these organisms represent Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria, allowing us to evaluate the differential susceptibility of these groups to the extract, which is a critical consideration given the structural differences in their cell walls [21,24]. Despite this rationale, the use of only two indicator organisms constitutes a limitation of this study. It is therefore recommended that future investigations evaluate the efficacy of the extract against a broader range of waterborne pathogens, including *Salmonella* spp., *V. cholerae*, *P. aeruginosa*, *Enterococcus* spp., and enteric viruses, in order to fully assess its potential for water antimicrobial activity. Such information would provide valuable insights for practical applications in real-world water treatment scenarios.

While the extract shows promising potential for decentralized water treatment, further research is required on scale optimization, long-term stability, comprehensive toxicology, and organoleptic impact before field implementation. We recognize that this study was based on a single extraction batch of *Citrus paradisi* peels and that reproducibility across batches was not evaluated, which represents a limitation of this research, since the chemical composition of plant extracts may vary depending on factors such as the geographical origin of the plant, harvest season, storage conditions, and processing parameters [15,16]. Therefore, before considering the extract for practical applications, reproducibility of extraction yield, chemical profile, and antimicrobial activity must be evaluated across multiple independent batches, as this is essential to ensure product consistency and robustness for potential industrial or practical applications [30,31].

4. Conclusions

This study demonstrated that the ethanolic extract obtained from *Citrus paradisi* peel constitutes an effective natural alternative for bacterial reduction in contaminated surface waters, with antimicrobial performance significantly superior to that of the extraction solvent and with a clear dependence on the applied concentration. Results obtained through the broth macrodilution method revealed that the extract exhibits differential bactericidal activity, being more potent against *S. aureus* (Gram-positive bacterium), attributable to structural differences in the cell walls of both bacterial groups.

Application of the extract in real aqueous matrices with varying levels of microbial contamination and turbidity validated its potential under near-real conditions, achieving removal percentages above 80% for both microorganisms (*Staphylococcus* spp. and *E. coli*) even under high contamination scenarios.

The novelty of this work lies in the specific validation of the extract for surface water bacterial reduction in real matrices, an application that has not been previously explored for *Citrus paradisi* extracts. Unlike laboratory-based antimicrobial studies, our approach addresses the practical challenges of water treatment in resource-limited settings, including the presence of organic matter, competing microflora, and variable turbidity.

Spectrophotometric characterization by FTIR and UV-Vis suggested the presence of functional groups and secondary metabolites (flavonoids, terpenes, and phenolic compounds) that are consistent with the observed antimicrobial activity. However, in the absence of chromatographic analysis (HPLC-MS), the specific compounds responsible for the effect and their mechanisms of action remain hypothetical and should be investigated in future studies.

Statistical analysis using ANOVA and Bonferroni post hoc tests confirmed the significance of the findings, demonstrating statistically significant differences ($p < 0.05$) between extract and ethanol treatments at all evaluated times, as well as between the two extract concentrations in most comparisons, particularly in highly contaminated water and against *E. coli*.

From an applied and sustainability perspective, this research is particularly relevant as it demonstrates the feasibility of valorizing an agro-industrial residue by transforming it into an input with potential to address a critical public health issue. The results align with the principles of circular economy and green chemistry, proposing a natural alternative that could reduce dependence on conventional disinfectants such as chlorine, whose use generates toxic by-products and has been associated with microbial resistance.

In summary, the ethanolic peel extract of *Citrus paradisi* emerges as a promising alternative for bacterial reduction in contaminated surface waters, with proven efficacy against Gram-positive and Gram-negative bacteria of sanitary relevance. The findings of this study contribute to the foundation for developing natural, accessible, and sustainable disinfectant formulations that may effectively support the achievement of SDG 6: ensuring availability and sustainable management of water and sanitation for all, particularly in communities with limited resources and restricted access to conventional water treatment technologies.

Author Contributions: Conceptualization, L.C.-C. and M.G.-C.; methodology, M.D.L.C.-N. and V.S.-A.; software, P.P.-P.; validation, L.C.-C., M.G.-C. and M.D.L.C.-N.; formal analysis, V.S.-A.; investigation, M.D.L.C.-N. and P.P.-P.; resources, L.C.-C.; data curation, M.G.-C.; writing—original draft preparation, L.C.-C. and M.D.L.C.-N.; writing—review and editing, M.G.-C. and V.S.-A.; visualization, P.P.-P.; supervision, L.C.-C.; project administration, M.G.-C.; funding acquisition, L.C.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors acknowledge the Research Centers and Institutes of Universidad César Vallejo for providing the facilities and institutional support for this study.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Appendix A.1

Table A1 shows that the distribution of removal data meets the assumptions of normality in most cases. For ethanol treatments, all p-values obtained from the Shapiro–Wilk tests were greater than 0.05, for both *Staphylococcus* spp. and *E. coli*, and at both contamination

levels. This indicates that the data from these groups fit a normal distribution, allowing the use of parametric statistical tests. Similarly, under high contamination conditions, all treatments with extract at 2.5 and 5.0 mg/mL presented p -values > 0.05 , also confirming normality in these more complex scenarios.

A relevant finding is that, in slightly contaminated water, extract treatments that achieved 100% removal did not allow calculation of p -values (N.A.), since the absence of variability prevents the normality test. This highlights the absolute and consistent efficacy of the extract, particularly at 5.0 mg/mL.

Table A1. Normality test applied to bacterial removal percentage data for *Staphylococcus* spp. and *E. coli* obtained during treatment with ethanol and *Citrus paradisi* extract at 2.5 and 5.0 mg/mL in processed water samples.

Extract Dosage	% Removal of <i>Staphylococcus</i> sp. (mean $\pm$ SD) in slightly contaminated water					
	6 h	p -value	12 h	p -value	24 h	p -value
Ethanol	5.01 $\pm$ 5.28	0.22	8.97 $\pm$ 3.83	0.13	14.37 $\pm$ 1.90	0.60
2.5 mg/mL	95.05 $\pm$ 2.07	0.36	100.00 $\pm$ 0.00	N.A.	100.00 $\pm$ 0.00	N.A.
5 mg/mL	100.00 $\pm$ 0.00	N.A.	100.00 $\pm$ 0.00	N.A.	100.00 $\pm$ 0.00	N.A.
Extract Dosage	% Removal of <i>E. coli</i> (mean $\pm$ SD) in slightly contaminated water					
	6 h	p -value	12 h	p -value	24 h	p -value
Ethanol	25.11 $\pm$ 2.51	0.22	25.45 $\pm$ 5.95	0.13	17.86 $\pm$ 5.46	0.60
2.5 mg/mL	90.64 $\pm$ 0.40	0.36	100.00 $\pm$ 0.00	N.A.	100.00 $\pm$ 0.00	N.A.
5 mg/mL	100.00 $\pm$ 0.00	N.A.	100.00 $\pm$ 0.00	N.A.	100.00 $\pm$ 0.00	N.A.
Extract Dosage	% Removal of <i>Staphylococcus</i> sp. (mean $\pm$ SD) in highly contaminated water					
	6 h	p -value	12 h	p -value	24 h	p -value
Ethanol	7.79 $\pm$ 2.66	0.71	15.07 $\pm$ 2.52	0.48	8.98 $\pm$ 4.38	0.17
2.5 mg/mL	76.40 $\pm$ 4.50	0.55	73.99 $\pm$ 6.76	0.88	71.99 $\pm$ 4.57	0.07
5 mg/mL	88.20 $\pm$ 2.84	0.25	84.71 $\pm$ 3.59	0.57	84.59 $\pm$ 2.94	0.06
Extract Dosage	% Removal of <i>E. coli</i> (mean $\pm$ SD) in highly contaminated water					
	6 h	p -value	12 h	p -value	24 h	p -value
Ethanol	0.81 $\pm$ 5.27	0.57	4.15 $\pm$ 5.31	0.11	7.40 $\pm$ 8.34	0.36
2.5 mg/mL	64.32 $\pm$ 6.18	0.31	63.28 $\pm$ 7.70	0.913	61.16 $\pm$ 6.79	0.69
5 mg/mL	80.02 $\pm$ 4.16	0.80	80.74 $\pm$ 3.23	0.07	81.61 $\pm$ 4.15	0.81

Note: N.A. = Not applicable, since results reported 100% removal due to absence of bacterial growth on plates.

Appendix A.2

The results of the Bonferroni test for slightly contaminated water (Table A2) demonstrate statistically significant differences ($p < 0.05$) in most comparisons between ethanol and extract treatments, as well as between extract concentrations. At 6 h, ethanol showed significantly lower efficacy ($p = 0.000$) compared to both extract concentrations for *Staphylococcus* spp. and *E. coli*, with mean differences exceeding 90 percentage points in some cases. A significant superiority of the 5.0 mg/mL extract over the 2.5 mg/mL extract was also observed against *E. coli* ($p = 0.022$), although this difference was not significant for *Staphylococcus* spp. ($p = 0.077$). At 12 and 24 h, both extract treatments achieved 100% removal for *Staphylococcus* spp., eliminating any significant differences between them ($p = 1.000$). However, ethanol remained significantly less effective ($p = 0.000$) at all times and for both microorganisms.

Regarding bacterial comparisons, the extract showed similar efficacy against *Staphylococcus* spp. and *E. coli* at higher concentrations and longer exposure times ($p = 1.000$). Nevertheless, at 6 h, significant differences were detected in favor of *Staphylococcus* spp. when compared with ethanol. These results confirm the marked superiority of the extract over ethanol and its concentration-dependent effectiveness, particularly evident during the initial hours of treatment.

Table A2. Bonferroni Multiple Comparison Test applied to bacterial removal percentage data for *Staphylococcus* spp. and *E. coli* in different treatments performed on slightly contaminated water samples.

Variable (Time)	Treatment Comparison		Mean Difference	Standard Deviation	p-Value
T6H	<i>Staphylococcus</i> sp.-Ethanol	<i>Staphylococcus</i> sp.-2.5 mg/mL	−90.03200 *	1.60848	0.000
		<i>Staphylococcus</i> sp.-5.0 mg/mL	−94.98600 *	1.60848	0.000
		<i>E. coli</i> -Ethanol	−20.09800 *	1.60848	0.000
		<i>E. coli</i> -2.5 mg/mL	−85.62400 *	1.60848	0.000
		<i>E. coli</i> -5.0 mg/mL	−91.39200 *	1.60848	0.000
	<i>Staphylococcus</i> sp.-2.5 mg/mL	<i>Staphylococcus</i> sp.-5.0 mg/mL	−4.95400	1.60848	0.077
		<i>E. coli</i> -Ethanol	69.93400 *	1.60848	0.000
		<i>E. coli</i> -2.5 mg/mL	4.40800	1.60848	0.171
		<i>E. coli</i> -5.0 mg/mL	−1.36000	1.60848	1.000
	<i>Staphylococcus</i> sp.-5.0 mg/mL	<i>E. coli</i> -Ethanol	74.88800 *	1.60848	0.000
		<i>E. coli</i> -2.5 mg/mL	9.36200 *	1.60848	0.000
		<i>E. coli</i> -5.0 mg/mL	3.59400	1.60848	0.525
	<i>E. coli</i> -Ethanol	<i>E. coli</i> -2.5 mg/mL	−65.52600 *	1.60848	0.000
		<i>E. coli</i> -5.0 mg/mL	−71.29400 *	1.60848	0.000
	<i>E. coli</i> -2.5 mg/mL	<i>E. coli</i> -5.0 mg/mL	−5.76800 *	1.60848	0.022
T12H	<i>Staphylococcus</i> sp.-Ethanol	<i>Staphylococcus</i> sp.-2.5 mg/mL	−91.03400 *	1.82754	0.000
		<i>Staphylococcus</i> sp.-5.0 mg/mL	−91.03400 *	1.82754	0.000
		<i>E. coli</i> -Ethanol	−16.48400 *	1.82754	0.000
		<i>E. coli</i> -2.5 mg/mL	−90.32600 *	1.82754	0.000
		<i>E. coli</i> -5.0 mg/mL	−91,03400 *	1.82754	0.000
	<i>Staphylococcus</i> sp.-2.5 mg/mL	<i>Staphylococcus</i> sp.-5.0 mg/mL	0.00000	1.82754	1.000
		<i>E. coli</i> -Ethanol	74.55000 *	1.82754	0.000
		<i>E. coli</i> -2.5 mg/mL	0.70800	1.82754	1.000
		<i>E. coli</i> -5.0 mg/mL	0.00000	1.82754	1.000
	<i>Staphylococcus</i> sp.-5.0 mg/mL	<i>E. coli</i> -Ethanol	74.55000 *	1.82754	0.000
		<i>E. coli</i> -2.5 mg/mL	0.70800	1.82754	1.000
		<i>E. coli</i> -5.0 mg/mL	0.00000	1.82754	1.000
	<i>E. coli</i> -Ethanol	<i>E. coli</i> -2.5 mg/mL	−73.84200 *	1.82754	0.000
		<i>E. coli</i> -5.0 mg/mL	−74.55000 *	1.82754	0.000
	<i>E. coli</i> -2.5 mg/mL	<i>E. coli</i> -5.0 mg/mL	−0.70800	1.82754	1.000
T24H	<i>Staphylococcus</i> sp.-Ethanol	<i>Staphylococcus</i> sp.-2.5 mg/mL	−85.63400 *	1.49331	0.000
		<i>Staphylococcus</i> sp.-5.0 mg/mL	−85.63400 *	1.49331	0.000
		<i>E. coli</i> -Ethanol	−3.49800	1.49331	0.417
		<i>E. coli</i> -2.5 mg/mL	−85.63400 *	1.49331	0.000
		<i>E. coli</i> -5.0 mg/mL	−85.63400 *	1.49331	0.000
	<i>Staphylococcus</i> sp.-2.5 mg/mL	<i>Staphylococcus</i> sp.-5.0 mg/mL	0.00000	1.49331	1.000
		<i>E. coli</i> -Ethanol	82.13600 *	1.49331	0.000
		<i>E. coli</i> -2.5 mg/mL	0.00000	1.49331	1.000
		<i>E. coli</i> -5.0 mg/mL	0.00000	1.49331	1.000
	<i>Staphylococcus</i> sp.-5.0 mg/mL	<i>E. coli</i> -Ethanol	82.13600 *	1.49331	0.000
		<i>E. coli</i> -2.5 mg/mL	0.00000	1.49331	1.000
		<i>E. coli</i> -5.0 mg/mL	0.00000	1.49331	1.000
	<i>E. coli</i> -Ethanol	<i>E. coli</i> -2.5 mg/mL	−82.13600 *	1.49331	0.000
		<i>E. coli</i> -5.0 mg/mL	−82.13600 *	1.49331	0.000
	<i>E. coli</i> -2.5 mg/mL	<i>E. coli</i> -5.0 mg/mL	0.00000	1.49331	1.000

Note: * Statistically significant at $p < 0.05$.

Appendix A.3

The results of the Bonferroni test for highly contaminated water (Table A3) reveal statistically significant differences ($p < 0.05$), highlighting the superiority of the extract over ethanol and the effect of concentration. At 6 h, both extract treatments (2.5 and 5.0 mg/mL) were significantly more effective than ethanol against *Staphylococcus* spp. ($p = 0.000$). Moreover, the 5.0 mg/mL concentration showed significantly greater efficacy than 2.5 mg/mL for the same bacterium ($p = 0.005$). For *E. coli*, a clear superiority of both extract concentrations over ethanol was also observed ($p = 0.000$), and the higher concentration (5.0 mg/mL) was significantly better than the lower one (2.5 mg/mL), with a mean difference of 56.53% ($p = 0.000$).

At 12 h, the trend persisted. The 5.0 mg/mL extract showed marginally higher efficacy than 2.5 mg/mL against *Staphylococcus* spp. ($p = 0.051$), a borderline value suggesting a tendency. Against *E. coli*, the superiority of 5.0 mg/mL over 2.5 mg/mL was again statistically significant ($p = 0.000$). At 24 h, the difference between the two extract concentrations became statistically significant once more for *Staphylococcus* spp. ($p = 0.021$), confirming the advantage of the higher dose over a prolonged period. In the case of *E. coli*, the superiority of 5.0 mg/mL over 2.5 mg/mL remained robust ($p = 0.000$) at all evaluated times.

In summary, under highly contaminated conditions, the 5.0 mg/mL extract consistently demonstrated greater efficacy than the 2.5 mg/mL extract, particularly against *E. coli*, underscoring the importance of dosage in achieving optimal removal in environments with high initial microbial loads.

Table A3. Bonferroni Multiple Comparison Test applied to bacterial removal percentage data for *Staphylococcus* spp. and *E. coli* in different treatments performed on highly contaminated water samples.

Variable (Time)	Treatment Comparison		Mean Difference	Standard Deviation	p-Value
T6H	<i>Staphylococcus</i> sp.-Ethanol	<i>Staphylococcus</i> sp.-2.5 mg/mL	−68.61200 *	2.81227	0.000
		<i>Staphylococcus</i> sp.-5.0 mg/mL	−80.41000 *	2.81227	0.000
		<i>E. coli</i> -Ethanol	6.97400	2.81227	0.308
		<i>E. coli</i> -2.5 mg/mL	−56.53400 *	2.81227	0.000
		<i>E. coli</i> -5.0 mg/mL	−72.23200 *	2.81227	0.000
	<i>Staphylococcus</i> sp.-2.5 mg/mL	<i>Staphylococcus</i> sp.-5.0 mg/mL	−11.79800 *	2.81227	0.005
		<i>E. coli</i> -Ethanol	75.58600 *	2.81227	0.000
		<i>E. coli</i> -2.5 mg/mL	12.07800 *	2.81227	0.004
		<i>E. coli</i> -5.0 mg/mL	−3.62000	2.81227	1.000
	<i>Staphylococcus</i> sp.-5.0 mg/mL	<i>E. coli</i> -Ethanol	87.38400 *	2.81227	0.000
		<i>E. coli</i> -2.5 mg/mL	23.87600 *	2.81227	0.000
		<i>E. coli</i> -5.0 mg/mL	8.17800	2.81227	0.116
	<i>E. coli</i> -Ethanol	<i>E. coli</i> -2.5 mg/mL	−63.50800 *	2.81227	0.000
		<i>E. coli</i> -5.0 mg/mL	−79.20600 *	2.81227	0.000
	<i>E. coli</i> -2.5 mg/mL	<i>E. coli</i> -5.0 mg/mL	56.53400 *	2.81227	0.000
T12H	<i>Staphylococcus</i> sp.-Ethanol	<i>Staphylococcus</i> sp.-2.5 mg/mL	−58.91800 *	3.29521	0.000
		<i>Staphylococcus</i> sp.-5.0 mg/mL	−69.63200 *	3.29521	0.000
		<i>E. coli</i> -Ethanol	10.92200 *	3.29521	0.044
		<i>E. coli</i> -2.5 mg/mL	−48.20400 *	3.29521	0.000
		<i>E. coli</i> -5.0 mg/mL	−65.66400 *	3.29521	0.000
	<i>Staphylococcus</i> sp.-2.5 mg/mL	<i>Staphylococcus</i> sp.-5.0 mg/mL	−10.71400	3.29521	0.051
		<i>E. coli</i> -Ethanol	69.84000 *	3.29521	0.000
		<i>E. coli</i> -2.5 mg/mL	10.71400	3.29521	0.051
		<i>E. coli</i> -5.0 mg/mL	−6.74600	3.29521	0.776
	<i>Staphylococcus</i> sp.-5.0 mg/mL	<i>E. coli</i> -Ethanol	80.55400 *	3.29521	0.000
		<i>E. coli</i> -2.5 mg/mL	21.42800 *	3.29521	0.000
		<i>E. coli</i> -5.0 mg/mL	3.96800	3.29521	1.000

Table A3. Cont.

Variable (Time)	Treatment Comparison		Mean Difference	Standard Deviation	p-Value
T24H	<i>E. coli</i> -Ethanol	<i>E. coli</i> -2.5 mg/mL	−59.12600 *	3.29521	0.000
		<i>E. coli</i> -5.0 mg/mL	−76.58600 *	3.29521	0.000
	<i>E. coli</i> -2.5 mg/mL	<i>E. coli</i> -5.0 mg/mL	−17.46000 *	3.29521	0.000
	<i>Staphylococcus</i> sp.-Ethanol	<i>Staphylococcus</i> sp.-2.5 mg/mL	−63.01600 *	3.47843	0.000
		<i>Staphylococcus</i> sp.-5.0 mg/mL	−75.60600 *	3.47843	0.000
		<i>E. coli</i> -Ethanol	1.57600	3.47843	1.000
		<i>E. coli</i> -2.5 mg/mL	−52.18200 *	3.47843	0.000
		<i>E. coli</i> -5.0 mg/mL	−72.63200 *	3.47843	0.000
	<i>Staphylococcus</i> sp.-2.5 mg/mL	<i>Staphylococcus</i> sp.-5.0 mg/mL	−12.59000 *	3.47843	0.021
		<i>E. coli</i> -Ethanol	64.59200 *	3.47843	0.000
		<i>E. coli</i> -2.5 mg/mL	10.83400	3.47843	0.071
		<i>E. coli</i> -5.0 mg/mL	−9.61600	3.47843	0.162
	<i>Staphylococcus</i> sp.-5.0 mg/mL	<i>E. coli</i> -Ethanol	77.18200 *	3.47843	0.000
		<i>E. coli</i> -2.5 mg/mL	23.42400 *	3.47843	0.000
		<i>E. coli</i> -5.0 mg/mL	2.97400	3.47843	1.000
	<i>E. coli</i> -Ethanol	<i>E. coli</i> -2.5 mg/mL	−53.75800 *	3.47843	0.000
		<i>E. coli</i> -5.0 mg/mL	−74.20800 *	3.47843	0.000
	<i>E. coli</i> -2.5 mg/mL	<i>E. coli</i> -5.0 mg/mL	−20.45000 *	3.47843	0.000

Note: * Statistically significant at $p < 0.05$.

References

1. Guijarro, A. Informe OMS-UNICEF 2000-22: Persisten las Desigualdades en el Acceso al Agua Potable y Saneamiento. Available online: <https://ongawa.org/informe-oms-unicef-2000-22-desigualdades-persistentes-en-el-acceso-al-agua-potable-y-saneamiento/> (accessed on 6 January 2026).
2. World Health Organization (WHO). United Nations Children’s Fund (UNICEF) 1 in 4 People Globally Still Lack Access to Safe Drinking Water—WHO, UNICEF. Available online: <https://www.who.int/news/item/26-08-2025-1-in-4-people-globally-still-lack-access-to-safe-drinking-water---who--unicef> (accessed on 6 January 2026).
3. World Health Organization (WHO). *United Nations Children’s Fund (UNICEF) Progress on Household Drinking-Water, Sanitation and Hygiene 2000–2024: Special Focus on Inequalities*; World Health Organization: Geneva, Switzerland, 2025.
4. United Nations. *UN-Water Summary Progress Update 2021: SDG 6—Water and Sanitation for All*; United Nations: New York, NY, USA, 2021.
5. Arnold, K. Water Pollution and Children’s Health. In *Textbook of Children’s Environmental Health*; Etzel, R.A., Landrigan, P.J., Eds.; Oxford University Press: Nueva York, NY, USA, 2024; pp. 362–377.
6. Cuenca, C.; Vera, Y.; Banchón, C. Salud pública y agua segura en Latinoamérica: Soluciones sostenibles frente al cambio climático. *Res. Soc. Dev.* **2025**, *14*, e8714649088. [\[CrossRef\]](#)
7. Mazzeo, C. América Latina a La Zaga En Estudios de Bacterias Resistentes En Agua. Available online: <https://www.scidev.net/america-latina/news/america-latina-a-la-zaga-en-estudios-de-bacterias-resistentes-en-agua/> (accessed on 6 January 2026).
8. Guillén-Ferraro, M.L.; Cordovéz-Martínez, M.d.C.; González-Romero, A.C.; Medina-Ramírez, G.E.; Mur-Caicedo, L.; Marcillo-Valencia, K.G. Bacterias multirresistentes en aguas de riego del río Chibunga, Chimborazo, Ecuador. *Investig. Desarro.* **2024**, *17*, 16–25. [\[CrossRef\]](#)
9. Khan, S.; Mumtaz, Z.A.; Khan, A.R.; Ahmad, N.; Alqahtani, D.A. A Review of Conventional and Non-Conventional Disinfection Methods for Treating Potable Water and Wastewater. *Int. J. Chem. React. Eng.* **2025**, *23*, 895–916. [\[CrossRef\]](#)
10. Cai, Y.; Sun, T.; Li, G.; An, T. Traditional and Emerging Water Disinfection Technologies Challenging the Control of Antibiotic-Resistant Bacteria and Antibiotic Resistance Genes. *ACS ES T Eng.* **2021**, *1*, 1046–1064. [\[CrossRef\]](#)
11. Gao, X.; Jia, S.; Ma, L.; Pan, Y.; Ye, L.; Zhang, X.-X.; Zhou, Q.; Li, A.; Shi, P. Prolonged Exposure to Environmentally Relevant Concentrations of Chlorine Induces Heritable Antimicrobial Resistance in Disinfection Residual *Pseudomonas aeruginosa*. *Environ. Sci. Technol.* **2025**, *59*, 3895–3905. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Nam, J.-H.; Yoo, J.S. Sublethal Sodium Hypochlorite Exposure: Impact on Resistance-Nodulation-Cell Division Efflux Pump Overexpression and Cross-Resistance to Imipenem. *Antibiotics* **2024**, *13*, 828. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Gomes, J.; Matos, A.; Gmurek, M.; Quinta-Ferreira, R.M.; Martins, R.C. Ozone and Photocatalytic Processes for Pathogens Removal from Water: A Review. *Catalysts* **2019**, *9*, 46. [\[CrossRef\]](#)

14. Qadri, A.; Alam, M. Drinking Water Treatment Using Advanced Technologies. Available online: <https://www.iscientific.org/wp-content/uploads/2024/03/19-IJCBS-24-25-14-19.pdf> (accessed on 6 January 2026).
15. Singh, R.; Das, R.; Sangwan, S.; Rohatgi, B.; Khanam, R.; Peera, S.K.P.G.; Das, S.; Lyngdoh, Y.A.; Langyan, S.; Shukla, A.; et al. Utilisation of Agro-Industrial Waste for Sustainable Green Production: A Review. *Environ. Sustain.* **2021**, *4*, 619–636. [CrossRef]
16. Casas Cardoso, L.; Cejudo Bastante, C.; Mantell Serrano, C.; Martínez de la Ossa, E.J. Application of Citrus By-Products in the Production of Active Food Packaging. *Antioxidants* **2022**, *11*, 738. [CrossRef] [PubMed]
17. Nath, A.; Das, K.; Dhal, G.C. Global Status of Agricultural Waste-Based Industries, Challenges, and Future Prospects. In *Agricultural Waste to Value-Added Products*; Springer: Singapore, 2023; pp. 21–45.
18. Anwar, I.; Saeed, U.; Hussain Bukhari, M.; Saeed, S. A Study of Antimicrobial Effects of Citrus Paradisi'Grape Fruit. Available online: https://pjmhsonline.com/2018/oct_dec/pdf/1454.pdf (accessed on 6 January 2026).
19. Hussien, F.H.; Al-Khafaf, A.H.; Al-Rawi, N.F. The Effect of Grapefruit Extracts on Causative Agents of Vaginitis among Women in Babylon Governorate, Iraq. *Med. J. Babylon.* **2025**, *22*, 426–431. [CrossRef] [PubMed]
20. Hamdi, A.; Horchani, M.; Jannet, H.B.; Snoussi, M.; Noumi, E.; Bouali, N.; Kadri, A.; Polito, F.; De Feo, V.; Edziri, H. In Vitro Screening of Antimicrobial and Anti-Coagulant Activities, ADME Profiling, and Molecular Docking Study of Citrus Limon L. and Citrus Paradisi L. Cold-Pressed Volatile Oils. *Pharmaceuticals* **2023**, *16*, 1669. [CrossRef] [PubMed]
21. Arsène, M.M.J.; Podoprigora, I.V.; Davares, A.K.L.; Razan, M.; Das, M.S.; Senyagin, A.N. Antibacterial Activity of Grapefruit Peel Extracts and Green-Synthesized Silver Nanoparticles. *Vet. World* **2021**, *14*, 1330–1341. [CrossRef] [PubMed]
22. Nguyen, T.T.P.; Hoang, B.N.; Huynh, X.P.; Bach, L.G. Research on the Extraction Conditions of Naringin, Antioxidant, Antibacterial Properties from Duong La Cam Pomelo Peel, Vietnam. *Nat. Prod. Commun.* **2024**, *19*, 1934578X241305287. [CrossRef]
23. Zihlif, M.A.; Afifi, F.; Sharab, A.; Maroof, P.; Abu-Odeh, A.; Alsayed, A.R. Antimicrobial potency of red and yellow varieties of grapefruit (*Citrus paradisi* Macfad) grown in Jordan. *Pharm. Pract.* **2024**, *22*, 19. [CrossRef]
24. Ibrahim, F.M.; Abdelsalam, E.; Mohammed, R.S.; Ashour, W.E.S.; Vilas-Boas, A.A.; Pintado, M.; El Habbasha, E.S. Polyphenol-Rich Extracts and Essential Oil from Egyptian Grapefruit Peel as Potential Antioxidant, Antimicrobial, and Anti-Inflammatory Food Additives. *Appl. Sci.* **2024**, *14*, 2776. [CrossRef]
25. Ogunjinmi, O.E.; Adegbola, P.I.; Ajayi, I.A.; Orike, E.L. Chemical Profiling of Citrus Fruit-Peel and Stem Extracts by Modified UHPLC-TOF-MS and Their Antiplasmodial, Antioxidant and Antimicrobial Activities. *Pharmacol. Res. Nat. Prod.* **2024**, *3*, 100051. [CrossRef]
26. Hoang, B.N.; Ngo, T.C.Q.; Tran, T.H.N.; Dang, D.N.; Tran, T.T.; Nguyen, Q.K.A.; Nguyen, Q.C.; Nguyen, H.K.N. Biological activity and pectin content in orange, lemon, tangerine, grapefruit and kumquat peels in Vietnam. *Asian J. Chem.* **2023**, *36*, 255–262. [CrossRef]
27. Yefanova, S.L.N.; Ouédraogo, J.C.W.; Ouédraogo, B.; Bonzi-Coulibaly, Y.L. The Use of Plants for Drinking Water Disinfection: Traditional Knowledge, Scientific Validation, Current Challenges and Prospects for the Future. In *From Traditional to Modern African Water Management*; Springer International Publishing: Cham, Switzerland, 2022; pp. 115–133.
28. Özcan, M.M.; Ghafoor, K.; Al Juhaime, F.; Uslu, N.; Babiker, E.E.; Mohamed Ahmed, I.A.; Almusallam, I.A. Influence of Drying Techniques on Bioactive Properties, Phenolic Compounds and Fatty Acid Compositions of Dried Lemon and Orange Peel Powders. *J. Food Sci. Technol.* **2021**, *58*, 147–158. [CrossRef] [PubMed]
29. Šafranko, S.; Ćorković, I.; Jerković, I.; Jakovljević, M.; Aladić, K.; Šubarić, D.; Jokić, S. Green Extraction Techniques for Obtaining Bioactive Compounds from Mandarin Peel (*Citrus unshiu* Var. Kuno): Phytochemical Analysis and Process Optimization. *Foods* **2021**, *10*, 1043. [CrossRef] [PubMed]
30. Hao, K.; Hu, W.; Hou, M.; Cao, D.; Wang, Y.; Guan, Q.; Zhang, X.; Wang, A.; Yu, J.; Guo, B. Optimization of Ultrasonic-Assisted Extraction of Total Phenolics from *Citrus aurantium* L. Blossoms and Evaluation of Free Radical Scavenging, Anti-HMG-CoA Reductase Activities. *Molecules* **2019**, *24*, 2368. [CrossRef] [PubMed]
31. Wei, J.; Tao, B.; Ye, Z.; Li, Y.; Zhou, Z. Ultrasound-Assisted Extraction of Carotenoids from Citrus peel by Olive Oil and Its Application in Functional Emulsions. *Ultrason. Sonochem.* **2025**, *122*, 107629. [CrossRef] [PubMed]
32. CLSI Standard M07; Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 12th ed. Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2024.
33. Gómez, P.; Lozano, C.; Benito, D.; Estepa, V.; Tenorio, C.; Zarazaga, M.; Torres, C. Characterization of Staphylococci in Urban Wastewater Treatment Plants in Spain, with Detection of Methicillin Resistant Staphylococcus aureus ST398. *Environ. Pollut.* **2016**, *212*, 71–76. [CrossRef] [PubMed]
34. Ghimire, B.; Pokherel, M.K.; Banjara, M.R.; Rijal, K.R.; Ghimire, P. Extended spectrum beta lactamase Escherichia coli in Bagmati River, Kathmandu valley. *J. Nepal Health Res. Counc.* **2024**, *21*, 672–679. [CrossRef] [PubMed]
35. Neisi, A.; Esteresh, A.; Takdastan, A.; Orooji, N. Removal of turbidity and coliform bacteria from karoan river water by natural coagulants aid (bread yeast) with PAC. In *Proceedings of the International Conference on Chemical, Environmental and Biological Sciences (CEBS-2015), Dubai, United Arab Emirates, 18–19 March 2015*; International Institute of Chemical, Biological & Environmental Engineering: Singapore, 2015.

36. Ramavandi, B. Treatment of Water Turbidity and Bacteria by Using a Coagulant Extracted from Plantago Ovata. *Water Resour. Ind.* **2014**, *6*, 36–50. [CrossRef]
37. Movasaghi, Z.; Rehman, S.; Ur Rehman, D.I. Fourier Transform Infrared (FTIR) Spectroscopy of Biological Tissues. *Appl. Spectrosc. Rev.* **2008**, *43*, 134–179. [CrossRef]
38. Tsiraki, M.I.; Yehia, H.M.; Elobeid, T.; Osaili, T.; Sakkas, H.; Savvaidis, I.N. Viability of and Escherichia coli O157:H7 and Listeria monocytogenes in a Delicatessen Appetizer (Yogurt-Based) Salad as Affected by Citrus Extract (Citrox®) and Storage Temperature. *Food Microbiol.* **2018**, *69*, 11–17. [CrossRef] [PubMed]
39. Cormier, J.; Scott, R.; Janes, M. Antibacterial Activity of Biosecur[®] Citrus Extract Surface Cleaner against Vibrio vulnificus. *Open Microbiol. J.* **2013**, *7*, 130–134. [CrossRef] [PubMed]
40. Guimarães, R.; Barros, L.; Barreira, J.C.M.; Sousa, M.J.; Carvalho, A.M.; Ferreira, I.C.F.R. Targeting Excessive Free Radicals with Peels and Juices of Citrus Fruits: Grapefruit, Lemon, Lime and Orange. *Food Chem. Toxicol.* **2010**, *48*, 99–106. [CrossRef] [PubMed]
41. Aouant, K.; Christodoulou, P.; Tsiaka, T.; Strati, I.F.; Cavouras, D.; Sinanoglou, V.J. Implementation of Instrumental Analytical Methods, Image Analysis and Chemometrics for the Comparative Evaluation of Citrus Fruit Peels. *Foods* **2025**, *14*, 4115. [CrossRef] [PubMed]
42. Mare, R.; Pujia, R.; Maurotti, S.; Greco, S.; Cardamone, A.; Coppoletta, A.R.; Bonacci, S.; Procopio, A.; Pujia, A. Assessment of Mediterranean Citrus Peel Flavonoids and Their Antioxidant Capacity Using an Innovative UV-Vis Spectrophotometric Approach. *Plants* **2023**, *12*, 4046. [CrossRef] [PubMed]
43. Sanches, V.L.; Cunha, T.A.; Viganó, J.; de Souza Mesquita, L.M.; Faccioli, L.H.; Breikreitz, M.C.; Rostagno, M.A. Comprehensive Analysis of Phenolics Compounds in Citrus Fruits Peels by UPLC-PDA and UPLC-Q/TOF MS Using a Fused-Core Column. *Food Chem. X* **2022**, *14*, 100262. [CrossRef] [PubMed]
44. Thenarasan, D.; Deepak Muthu, S.; Nithyapriya, R.; Mohamed Nowfal, A.; Prabhu, T.; Sasikala, S. Quantitative analysis of phenolic compounds in citrus peels using UV-visible spectrophotometry. *Int. J. Pharm. Sci.* **2025**, *3*, 4138–4143. [CrossRef]
45. Al-Otibi, F.; Alkhudhair, S.K.; Alharbi, R.I.; Al-Askar, A.A.; Aljowaie, R.M.; Al-Shehri, S. The Antimicrobial Activities of Silver Nanoparticles from Aqueous Extract of Grape Seeds against Pathogenic Bacteria and Fungi. *Molecules* **2021**, *26*, 6081. [CrossRef] [PubMed]
46. Wongsu, P.; Phatikulrungsun, P.; Prathumthong, S. FT-IR Characteristics, Phenolic Profiles and Inhibitory Potential against Digestive Enzymes of 25 Herbal Infusions. *Sci. Rep.* **2022**, *12*, 6631. [CrossRef] [PubMed]
47. Yilmaz, H.; Gultekin Subasi, B.; Celebioglu, H.U.; Ozdal, T.; Capanoglu, E. Chemistry of Protein-Phenolic Interactions toward the Microbiota and Microbial Infections. *Front. Nutr.* **2022**, *9*, 914118. [CrossRef] [PubMed]
48. Stasiuk, M.; Kozubek, A. Biological Activity of Phenolic Lipids. *Cell. Mol. Life Sci.* **2010**, *67*, 841–860. [CrossRef] [PubMed]
49. Trombetta, D.; Castelli, F.; Sarpietro, M.G.; Venuti, V.; Cristani, M.; Daniele, C.; Saija, A.; Mazzanti, G.; Bisignano, G. Mechanisms of Antibacterial Action of Three Monoterpenes. *Antimicrob. Agents Chemother.* **2005**, *49*, 2474–2478. [CrossRef] [PubMed]
50. Shonekan, O.O.; Otuka, A.C.; Adeyemi, D.K.; Fatunsin, O.T. A Comparison of Antioxidant and Fourier Transform Infrared Spectroscopy (FTIR) Analysis on Extracts of *Syzygium guineenses* (Myrtaceae). Available online: <https://www.jbspr.com/wp-content/uploads/2020/01/1-7-Shonekan-et-al.-Antioxidant-and-FTIR-analysis-of-S.-guineenses-Final-Vol.1Issue2ad.pdf> (accessed on 22 December 2025).
51. Almutairy, B. Flavonoid-Mediated Biofilm Inhibition and Toxicological Evaluation of Atriplex Laciniata against Multidrug-Resistant MRSA. *Front. Pharmacol.* **2025**, *16*, 1577052. [CrossRef] [PubMed]
52. Ciriminna, R.; Petri, G.L.; Angellotti, G.; Luque, R.; Fabiano Tixier, A.-S.; Meneguzzo, F.; Pagliaro, M. Citrus Flavonoids as Antimicrobials. *Chem. Biodivers.* **2025**, *22*, e202403210. [CrossRef] [PubMed]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.