



# Essential Oils as Alternatives to Chlorine in the Postharvest Treatment of 'Dalhari' Watery Rose Apple Fruit

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## ABSTRACT

The thin skin and high water and sugar content of the watery rose apple cv. 'Dalhari' (*Syzygium aqueum*) make it highly susceptible to microbial infections. The fruit's skin serves as its outer protective layer, shielding it from mechanical damage and microbial invasion. Washing is the first step in postharvest fruit treatment. However, concerns over the potential carcinogenic effects of chlorine residues have prompted a need to reconsider its use in industrial washing processes. This study investigated alternative natural sanitizers for washing the 'Dalhari' watery rose apple, focusing on the use of essential oils. Five essential oils, betel, clove, cinnamon bark, lemon, and vanilla, were tested for their antimicrobial properties using the minimum inhibitory concentration (MIC) method. The active compounds in these essential oils were identified using GC-MS analysis. Following treatment with the essential oils, several parameters were assessed on the watery rose apple, including microbial population, weight loss, fruit firmness, total titratable acidity, total soluble solids, and pH levels. The results showed that a 0.1% betel essential oil washing treatment was as effective as a 20 ppm chlorine treatment. These findings suggest that essential oils, particularly betel oil, can be viable natural alternatives to chlorine for washing the watery rose apple cv. 'Dalhari.'

## Introduction

The watery rose apple cv. 'Dalhari' (*Syzygium aqueum*) is a highly valued native germplasm in Sleman Regency, known for its juicy flesh, sweetness, tenderness, thick flesh, and lower seed count compared to other watery rose apple cultivars (Septyani and Santoso, 2013). However, it has a limited shelf life due to its susceptibility to microbial contamination. Its delicate skin and high sugar content create an ideal environment for microbial growth (Setiawan et al., 2019).

Pectinolytic bacteria, in particular, can break down pectin, a crucial component of fruits, leading to a decline in quality and safety, making the fruit unfit for consumption (Jennylynd B. et al., 2011).

Traditionally, postharvest treatments at the farm level have been limited to washing the fruit with plain water. Inorganic substances like chlorine, chlorine dioxide, and hydrogen peroxide have also been used to reduce microbial contamination in fruits and vegetables (Calonico et al., 2019).

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However, prolonged use of these chemicals is discouraged due to potential health risks from residues. A study on lettuce washing showed a reduction in chlorine concentration from 1.5 mg L<sup>-1</sup> to 0.8 mg L<sup>-1</sup>, indicating that chlorine might still be present on equipment or produce after washing (Calonico et al., 2019). While chlorine-based compounds are still widely used in industrial washing, especially for preventing cross-contamination during batch washing (Gil et al., 2016, 2019), they can react with organic matter to form harmful disinfection by-products (DBPs) such as trihalomethanes (THMs) and haloacetic acids (HAAs) (Gadelha et al., 2019).

A study in Taiwan found high concentrations of dibromochloromethane and dichloroacetic acid in ready-to-eat vegetables, highlighting the risks of DBPs (Lin and Tsai, 2023). Prolonged exposure to THMs and HAAs is associated with health risks such as bladder cancer, reproductive issues, and colorectal cancer (Rahman et al., 2010; Parvez et al., 2019; Evlampidou et al., 2020). Beyond health concerns, chlorine also negatively impacts ecosystems. Chlorine residues in the environment can disrupt soil microbial communities and decrease the activity of crucial enzymes like urease, catalase, and phosphatase, which play a key role in nutrient cycling, potentially stunting plant growth (Song et al., 2019).

Given these issues, there is a growing interest in natural, eco-friendly alternatives like essential oils (EOs) for washing fruits and vegetables. EOs have demonstrated antibacterial properties, and their potential for postharvest applications in fruits and vegetables has been explored in various studies (Bilcu et al., 2014; Mandal and Mandal, 2015; Valdés et al., 2017; Utama et al., 2020). This study investigates the use of essential oils as a natural washing agent to eliminate microorganisms from watery rose apple cv. 'Dalhari'. Specifically, it aims to identify the most effective type and concentration of essential oil needed to inhibit microbial growth during postharvest washing of the fruit.

## Material and Methods

### *Selection of watery rose apple fruit*

Watery rose apple cv. 'Dalhari' fruits were harvested from local gardens in Berbah Subdistrict, Sleman District, Special Region of Yogyakarta, Indonesia (7.8046° S, 110.4436° E). Fruit maturity was determined using specific criteria, including a harvest period of 60-70 days after blooming, a combination of dark red skin and white flesh, a soluble solid content of 10 ± 1%, and a weight ranging from approximately 70-100 g. The fruits were detached from their stems

and plastic wrapping before being transported to the laboratory. They were carefully packed in cardboard containers and wrapped in paper to prevent damage during transport.

Upon arrival at the laboratory, the fruits were sorted based on uniform size and checked to ensure they showed no visible signs of damage or disease. The selected fruits were then stored at 25 °C (GEA 2dDEXPO-800AH / CN, Indonesia) for further observation.

### *Quantitative analysis of essential oils compounds by GC-MS*

The antimicrobial activity of the EO fractions was analyzed using gas chromatography-mass spectrometry (GC-MS) to investigate their chemical composition (Hajji et al., 2010). Essential oils of vanilla, cinnamon, betel, clove, and lemon were extracted using the cold-pressed method and obtained from Happy Green Garden, Indonesia. The analysis was conducted using a Thermo Scientific Chromeleon™ Dionex 7.2.8.10783 system.

### *Determination of optimal concentrations of essential oil*

Prior to administering the washing procedure, the optimal concentration of each essential oil (EO) was determined through minimum inhibitory concentration (MIC) analysis on isolated spoilage microorganisms, including bacteria, yeast, and fungi (Othman et al., 2011). The initial phase involved isolating putrefactive bacteria, yeasts, and fungi from watery rose apple fruit. EO inhibitory testing was then performed using the paper disk diffusion and pour plate methods.

### *Isolation of spoilage microorganisms*

To prepare dilution water, the water was sterilized in an autoclave (Hirayama HVE-50, Japan) for 45 minutes at 121 °C and 1 atm of pressure. One gram of watery rose apple fruit was then crushed and mixed with the sterilized dilution water (Othman et al., 2011). The dilution process involved sequentially diluting the solution at a 1:10 ratio, resulting in a 10-fold dilution from 10<sup>-2</sup> to 10<sup>-9</sup>. Afterward, 0.1 mL of each diluted sample was added to sterilized Potato Dextrose Agar (PDA, Merck, Germany) and Plate Count Agar (PCA, Merck, Germany), which were prepared by autoclaving at 121 °C and 1 atm pressure for 45 minutes.

The samples were incubated at 32 °C for 48 hours. Microorganisms that proliferated on PDA and PCA were identified as fungi, yeast, or bacteria. The selected microorganisms were isolated and transferred to separate Petri dishes: bacterial

colonies were cultured on PCA, while fungal and yeast colonies were grown on PDA. The isolated bacteria were further transferred using the streak-plate technique and cultured for subsequent microbiological analysis.

#### ***Paper disk inhibition test***

The assay for inhibiting spoilage microbes was conducted using the Kirby-Bauer disk diffusion susceptibility test (Hudzicki, 2016). Essential oils of vanilla, cinnamon, betel, clove, and lemon were extracted using the cold-press method and purchased from Happy Green Garden in Indonesia. Paper disks with a 1.2 cm diameter were treated with 0.1 mL of varying concentrations of each EO solution. The disks were then placed on three different Petri dishes: one containing Plate Count Agar (PCA) for bacterial inoculation, and two with Potato Dextrose Agar (PDA) for fungal and yeast inoculation. The Petri dishes were inverted and incubated for 48 hours at 32 °C. Inhibition zones around each disk were measured using calipers in millimeters, and each measurement was performed in triplicate.

#### ***Pour plate inhibition test***

The selected microbial colonies were inoculated into growth media containing varying concentrations of EO using the pour plate method. From each culture, 1 mL was transferred into a Petri dish containing the appropriate growth media, bacterial colonies were cultured in Plate Count Agar (PCA), while fungi and yeast were cultured in Potato Dextrose Agar (PDA). The Petri dish was gently swirled in a circular motion to ensure even distribution of the microorganisms. After incubation, the microbial colonies were counted, and the concentration of EO that resulted in the lowest microbial growth was identified as the optimum concentration for antimicrobial effectiveness.

#### ***Effects of essential oils washing treatment on watery rose apple fruit cv. 'Dalhari'***

The washing solution was prepared by mixing water, EO, and Tween 80. The concentration of EO was set to the optimal value determined by the minimum inhibitory concentration (MIC) analysis, while Tween 80 (Polysorbate 80) was added at 10% of the EO concentration. All components were blended in a mixer (Miyako HM-620) to create a homogenous washing emulsion (Kang and Song, 2018). For control treatments, plain water and a chlorine solution were used. The chlorine-washing solution was prepared by adding 0.02 mL of sodium

hypochlorite (Bratachem, Indonesia) to 1 L of water. The watery rose apple cv. 'Dalhari' fruits were soaked in the washing solution for 3 min, dried, wrapped in low-density polyethylene plastic (0.01-0.02 mm thickness), and stored at ambient temperature for further observation.

#### ***Microbial analysis***

A microbiological test was conducted to determine the total microorganisms using the plate count method on Plate Count Agar (PCA). After the washing treatment, observations were made on days 0, 3, 6, 9, 12, and 15. One g of watery rose apple cv. 'Dalhari' was placed in a test tube and diluted in 9 mL of distilled water. This solution was then subjected to a series of 10-fold dilutions until reaching a dilution level of  $10^{-6}$ . From the  $10^{-4}$  to  $10^{-6}$  dilution levels, 0.1 mL of the solution was taken and streaked onto Petri dishes containing PCA. The Petri dishes were incubated for 48 h at 32 °C in an inverted position. The number of colonies was subsequently counted using a colony counter (Setiawan et al., 2019).

#### ***Weight loss and fruit hardness measurements***

Weight loss measurements were conducted daily for 15 d using an electronic balance (OHAUS Scout® Pro, USA). The weight loss percentage was calculated by determining the difference between the initial and final weights of the fruits. Fruit hardness was assessed using a penetrometer (Lutron FR-5120, Lutron Electronic Enterprise Co., Ltd.), with the instrument's tip being jabbed three times at different locations on each fruit. Hardness measurements were taken every three days using a tip diameter of 3 mm. The force required to penetrate the fruit was automatically displayed on the penetrometer when recording the hardness value.

#### ***Total titratable acid, total soluble solid, and pH measurements***

Total titratable acid (TTA) analysis was performed to measure the total organic acid content in the sample solution. The analysis followed the titration method (AOAC, 1990), where one g of mashed watery rose apple was diluted in 100 mL of distilled water. Then, 10 mL of this solution was mixed with 1-3 drops of phenolphthalein indicator. Titration was carried out using 0.1N NaOH until the solution turned a stable pink color. Total soluble solids (TSS), or total dissolved solids, were measured to assess the concentration of organic components in the fruit. The TSS value was determined using a

handheld refractometer (ATAGO Pal-3, ATAGO Co., LTD, USA). The fruit was crushed to extract the juice, and a small amount of the liquid was placed on the lens of the refractometer. The indicated value corresponded to the total amount of dissolved solids. The pH value was measured using a pH meter (HANNA Instruments HI 8314, UK). Before use, the pH electrode was calibrated with a buffer solution, rinsed with distilled water, and dried. One g of pulverized watery rose apple fruit was mixed with 5 mL of purified water, and the electrode was immersed in this mixture. The pH value was recorded once a stable reading was displayed. TTA, TSS, and pH analyses were conducted at three-day intervals over a period of 15 d.

### Statistical analysis and data visualization

All statistical analyses in this study were conducted using R and RStudio (version 2023.09.0) for Windows. The 'agricolae' package was employed to analyze the mean significant

differences among observed parameters using Duncan's multiple range test ( $P < 0.05$ ). Data visualization was accomplished with the 'ggplot2' and 'cowplot' packages in RStudio.

## Results

### Major compounds in essential oils

EOs of betel, cinnamon bark, cloves, lemon, and vanilla were analyzed using gas chromatography-mass spectrometry (GC-MS) to identify their predominant chemical compositions (Fig. 1). Each EO exhibited distinct compositions of key compounds. However, both betel and clove EOs contained 3-Allyl-6-methoxyphenol and 3-Allyl-6-methoxyphenol acetate, though at different concentrations. Betel EO had a higher concentration of 3-Allyl-6-methoxyphenol acetate, while clove EO had a greater proportion of 3-Allyl-6-methoxyphenol. Approximately 50% of lemon EO and vanilla EO consisted primarily of a single component, D-limonene and ethyl vanillin, respectively.

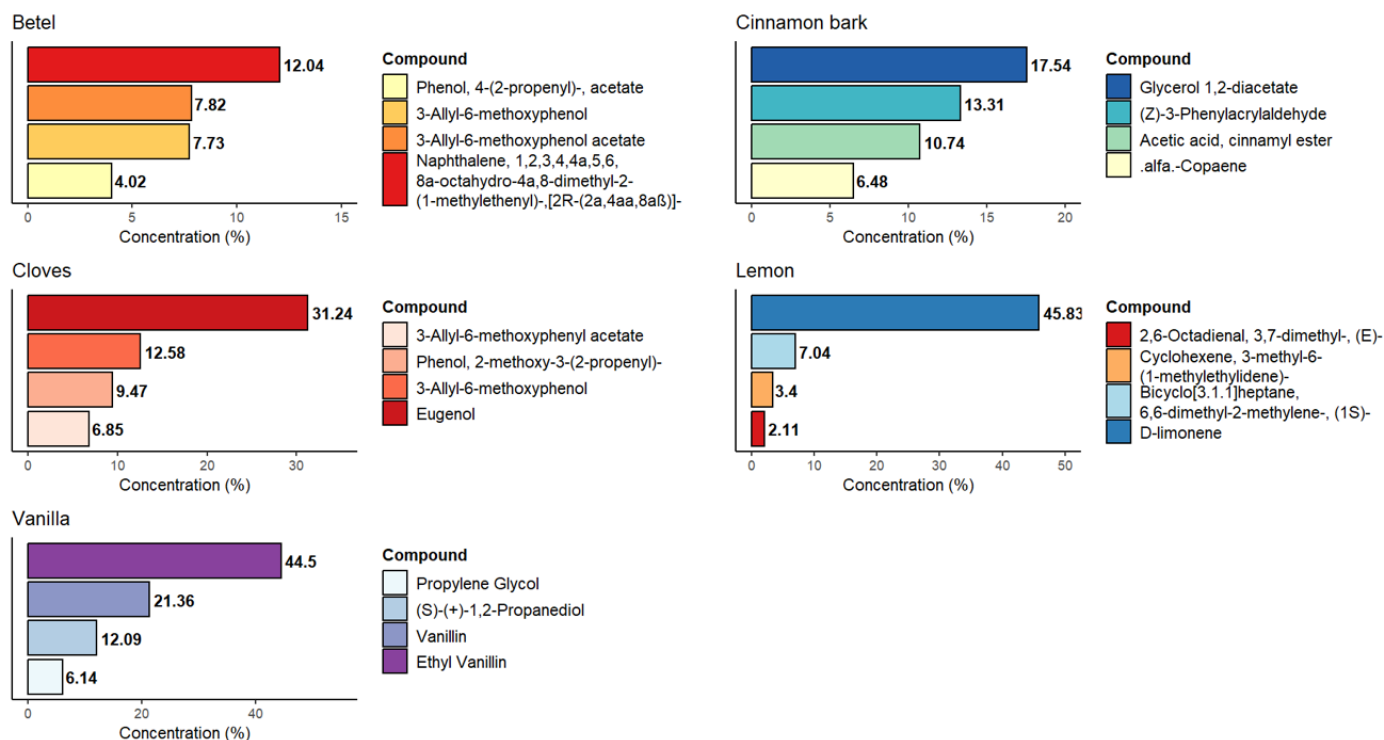


Fig. 1. Qualitative results for compounds in selected essential oils using GC-MS analysis.

### Determination of optimal concentration for each essential oil

Table 1 displays the features of each isolated pathogen, bacteria, fungi, and yeast found in the watery rose apple cv. 'Dahari'.

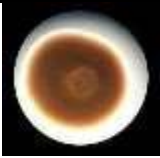


The optimal concentrations of each EO were subsequently applied in the washing treatment analysis. Table 2 shows that each EO exhibited an

ideal concentration for effectively inhibiting the selected spoilage microbes, as determined by the minimal inhibitory concentration study using the paper disk and pour method. The paper disk test results indicate that the most effective concentrations were 0.8% lemon EO (with inhibition zones of 2.4–4.4 mm), 0.1% betel EO (with inhibition zones of 3.5–6.0 mm), 0.7% clove EO (with inhibition zones of 2.4–2.8 mm), 0.5%

cinnamon EO (with inhibition zones of 4.1–20.6 mm), and 0.6% vanilla EO (with inhibition zones of 2.2–5.5 mm). These optimal concentrations

were then employed in further washing treatment analysis.

**Table 1.** Isolated pathogenic microorganisms of watery rose apple cv. 'Dahari.'

Identification	Bacteria	Fungi	Yeast
Microscopic observation			
Color	Yellow	Greyish white	White
Diameter	2 mm	1.5 mm	2.5 mm
Colony shape	Circular	Filamentous	Circular
Edges shape	Entire	Ramose	Crenate
Internal structure	Translucent	Filamentous	Finely Granular
Elevation	Low convex	Effuse	Effuse
Respiration type	Aerobe	Aerobe	Aerobe

**Table 2.** Minimal inhibitory concentration analysis using paper disk and pour-plate method expressed in inhibition zones and colony forming units.

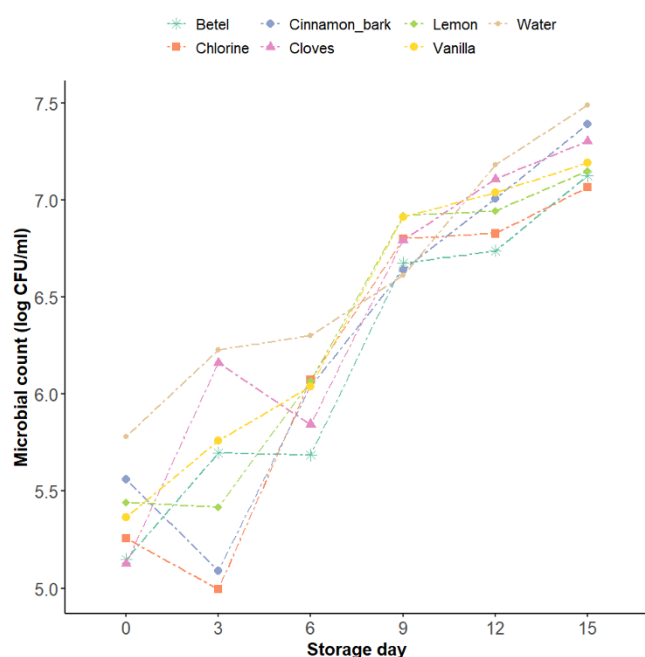
Essential oil	Concentration (%)	Inhibition zones (mm)			Colony Forming Unit (CFU)		
		Bacteria	Yeast	Fungi	Bacteria	Yeast	Fungi
Cinnamon	0.30	1.61 <sup>e</sup>	0.15 <sup>d</sup>	0.68 <sup>c</sup>	sp	5	20
	0.50	2.06 <sup>d</sup>	0.41 <sup>c</sup>	0.74 <sup>b</sup>	0	0	0
	0.70	2.50 <sup>c</sup>	0.52 <sup>b</sup>	0.76 <sup>b</sup>	0	0	0
	0.90	3.98 <sup>b</sup>	0.55 <sup>b</sup>	0.90 <sup>a</sup>	0	0	0
	1.10	7.00 <sup>a</sup>	0.63 <sup>a</sup>	0.92 <sup>a</sup>	0	0	0
Vanilla	0.20	0.16 <sup>b</sup>	0.133 <sup>c</sup>	0.27 <sup>d</sup>	sp	58	sp
	0.40	0.19 <sup>b</sup>	0.23 <sup>b</sup>	0.44 <sup>c</sup>	0	7	40
	0.60	0.24 <sup>b</sup>	0.26 <sup>ab</sup>	0.55 <sup>b</sup>	0	0	50
	0.80	0.53 <sup>a</sup>	0.29 <sup>ab</sup>	0.57 <sup>b</sup>	0	0	0
	1.00	0.20 <sup>b</sup>	0.35 <sup>a</sup>	0.75 <sup>a</sup>	0	0	0
Clove	0.30	0.16 <sup>e</sup>	0.16 <sup>c</sup>	0.23 <sup>c</sup>	sp	5	26
	0.50	0.22 <sup>d</sup>	0.21 <sup>b</sup>	0.25 <sup>c</sup>	0	2	3
	0.70	0.24 <sup>c</sup>	0.25 <sup>b</sup>	0.28 <sup>c</sup>	0	0	0
	0.90	0.33 <sup>b</sup>	0.24 <sup>b</sup>	0.54 <sup>b</sup>	0	0	0
	1.10	0.44 <sup>a</sup>	0.33 <sup>a</sup>	0.67 <sup>a</sup>	0	0	0
Betel	0.08	0.54 <sup>c</sup>	0.18 <sup>d</sup>	0.35 <sup>d</sup>	15	3	45
	0.10	0.60 <sup>c</sup>	0.35 <sup>c</sup>	0.58 <sup>c</sup>	0	1	0
	0.30	0.84 <sup>b</sup>	0.38 <sup>bc</sup>	0.74 <sup>b</sup>	0	0	0
	0.50	0.84 <sup>b</sup>	0.43 <sup>b</sup>	0.88 <sup>a</sup>	0	0	0
	0.70	1.00 <sup>a</sup>	0.55 <sup>a</sup>	0.91 <sup>a</sup>	0	0	0
Lemon	0.60	0.26 <sup>c</sup>	0.18 <sup>d</sup>	0.16 <sup>d</sup>	0	3	4
	0.80	0.44 <sup>b</sup>	0.32 <sup>c</sup>	0.24 <sup>c</sup>	0	1	0
	1.00	0.46 <sup>b</sup>	0.36 <sup>b</sup>	0.59 <sup>b</sup>	0	0	0
	1.20	0.55 <sup>a</sup>	0.41 <sup>a</sup>	0.76 <sup>a</sup>	0	0	0
	1.40	0.55 <sup>a</sup>	0.42 <sup>a</sup>	0.77 <sup>a</sup>	0	0	0

Same alphabetic orders indicate no significant difference between the values within the same essential oil types in different concentrations after Duncan's Multiple Range Test ( $P < 0.05$ ).



### Effects of different essential oils as washing material in watery rose apple fruit

Multiple variables were used to evaluate the effects of different EOs as eco-friendly alternatives to traditional washing agents on watery rose apple fruit cv. 'Dalhari'. Figure 2 illustrates the surface microbial count of the fruit

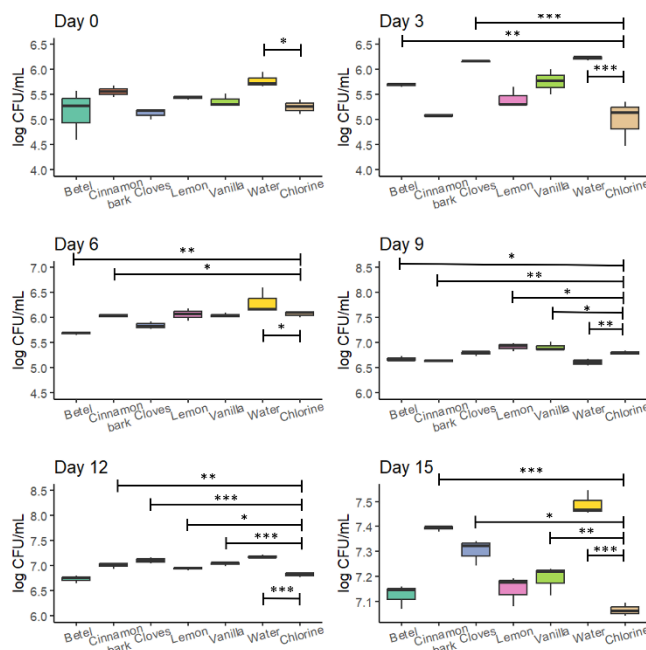


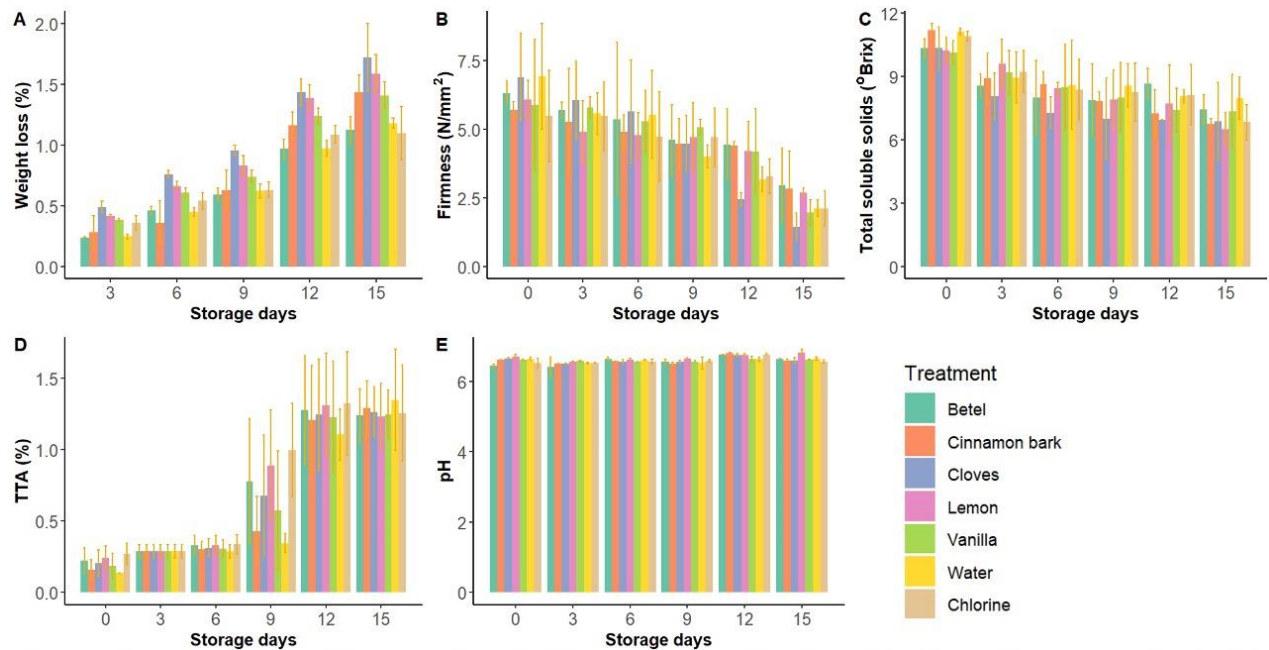
**Fig. 2.** Surface microbial load of watery rose apple fruit treated with different washing treatments. Asterisks indicate statistically significant differences in microbial load between chlorine and other treatments (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P = 0$ ).

The population of microorganisms in this study exhibited an upward trend over the 15-day storage period, with microbial load beginning to increase around day 9 and continuing through day 15. The type of EO influenced bacterial proliferation. On the final day, samples washed with plain water had the highest concentration of microorganisms, while those treated with chlorine had the lowest. The washing treatments with betel ( $P = 0.15$ ) and clove ( $P = 0.05$ ) EOs showed no statistically significant differences compared to the chlorine treatment. This study also evaluated weight loss, firmness, total soluble solids, total titratable acidity, and pH in watery rose apple fruit cv. 'Dalhari' to assess the effects of different washing methods. These parameters were measured at three-day intervals over a 15-day storage period (Fig. 3).

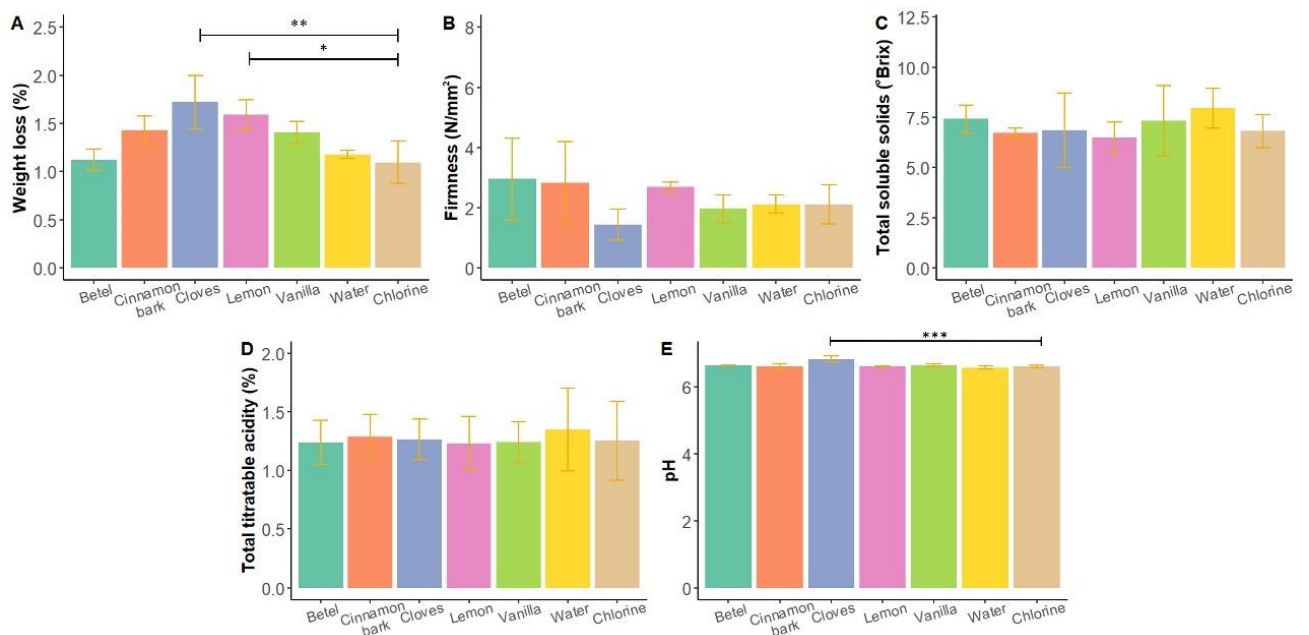
The weight loss graph for watery rose apples during storage showed a general increase across all treatments (Fig. 3A). Samples washed with clove essential oil exhibited the highest percentage of weight reduction throughout the observation period compared to other essential

oils. Figure 3B demonstrates a decline in fruit firmness over time, while Figure 3C reveals a decreasing trend in TSS. The graph depicting TTA showed a positive correlation with the duration of storage (Fig. 3D). According to Figure 3E, pH levels remained consistent throughout the storage period. Figure 4 presents a comparative analysis of weight loss, firmness, TSS, TTA, and pH of watery rose apple fruit cv. 'Dalhari' on the final day of observation, following treatments with various essential oils. The study aimed to explore alternative solutions to chlorine for industrial use. On day 15, notable differences were observed in weight reduction and pH between the chlorine and essential oil treatments. Compared to clove essential oil, chlorine treatment resulted in significantly lower weight loss ( $P = 0.0046$ ) and a lower pH level ( $P = 0.00026$ ). Chlorine also produced a lower percentage of weight reduction than lemon essential oil ( $P = 0.0186$ ). Figure 4 further shows no significant differences in firmness, TSS, and TTA across the different washing methods.





**Fig. 3.** Changes in weight loss (A), firmness (B), total soluble solids (C), total titratable acidity (D), and pH (E) of watery rose apple fruit with different washing treatments after 15 d of storage.



**Fig. 4.** Changes in weight loss (A), firmness (B), total soluble solids (C), total titratable acidity (D), and (E) pH of watery rose apple fruit with different essential oil washing treatments at d 15. Asterisks indicate statistically significant differences among observed parameters between chlorine and other treatments (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P = 0$ ).

## Discussion

Fruit surfaces are favorable habitats for the proliferation of microorganisms, especially pathogenic agents. Reported incidents of illness linked to fresh-cut food experienced a substantial increase in 2015. The occurrence of these

epidemics was ascribed to the rapid increase in a number of disease-causing microorganisms on the visible areas of fruits and vegetables (Callejón et al., 2015). Thus, a proficient cleansing technique is a crucial operation that greatly influences the length of time an item can be stored

and its level of safety. This study examined various EOs to determine their potential as a natural substitute for chlorine in the process of washing fruits. When compared to ordinary water, the EO treatments exhibited a substantial reduction in the growth of surface microbes on watery rose apple fruit cv. 'Dalhari' (Fig. 2). EOs can contain many molecules that possess antibacterial effects. Betel oil is composed of significant phytochemical ingredients including piperine, chavicol, hydroxychavicol, chavibetol, allyl pyrocatechol, carvacrol, terpinene, cineole, cadinene, eugenol, and other phenolic compounds. These chemicals have been scientifically demonstrated to have inhibitory effects on fungus and bacteria (Patel and Jasrai, 2013). Phenol acts as an antibacterial by causing harm to cell walls, causing precipitation of cell proteins in bacteria, and poisoning the protoplasm (Fitriyani et al., 2011). Cinnamaldehyde, also known as (Z)-3-phenylacrylaldehyde, is a prominent constituent of cinnamon essential oil. The chemical has the ability to hinder the activity and proliferation of fungus and bacteria (Yulianto et al., 2012). Eugenol, a primary component of clove essential oil, has the ability to infiltrate the cell and cytoplasm membrane, leading to cell leakage and the death of bacteria and fungi (Shabnam et al., 2012). Lemon essential oil mostly comprises limonene molecules, which are monoterpene compounds capable of binding to the cell plasma membrane. This binding leads to the disruption of membrane integrity and proton transport, hence reducing the growth of fungus and bacteria (Espina et al., 2013). Previous studies on inactivation of *E. coli* by terpenes and other terpenoids (such as carvacrol or citral) have shown sub-lethal damage in the outer membrane and cytoplasm, showing membrane disruption as an inactivation mechanism of these compounds (Espina et al., 2013). Research by Cava-Roda et al. (2021) showed vanillin antimicrobial properties against *Listeria monocytogenes* and *E. Coli O157:H7* (Cava-Roda et al., 2012). The bactericidal activity of vanillin on *E. Coli O157:H7* is attributed to its ability to cause membrane damage and alter energy metabolism. According to Figure 2, all washing treatments on the final day of observation had a surface microbial load value exceeding  $7 \log \text{CFU g}^{-1}$ . The application of chlorine, lemon, and betel essential oil on watery rose apple fruit did not result in any noticeable variation ( $P > 0.05$ ) in surface bacteria. Therefore, it may be inferred that lemon and betel essential oils, when used as washing agents, are equally effective as chlorine in eliminating surface germs on watery rose apple fruit cv. 'Dalhari'. Following

the harvest, fruit commodities undergo various physicochemical changes. These alterations occurring after harvest indicate the duration the fruit can be stored without spoilage. In addition to respiration rate, fruit deterioration can be influenced by other factors, such as microbial infection (Fang and Wakisaka, 2021). The current investigation assessed various time-dependent physicochemical characteristics to determine the shelf-life of watery rose apple fruit cv. 'Dalhari,' following different sanitization procedures. Figure 3 demonstrates that the watery rose apple fruit had increased weight loss and total soluble solids, a decrease in hardness, and total titratable acidity after a 15-d storage period, regardless of the various washing methods applied. On day 15, the weight loss of betel, cinnamon bark, and vanilla EOs was statistically similar to that of chlorine (Fig. 4). Watery rose apple cv. 'Dalhari' is a type of fruit with an inseparable exocarp and mesocarp due to the very thin layer of exocarp. When the exocarp of watery rose apple is damaged, the mesocarp is also affected (Lu and Lin, 2012). Microbial infection can result in enhanced ethylene production (Ravanbakhsh et al., 2018). The positive correlation between ethylene production and microorganism population was also reported in Kinnow mandarin (*Citrus nobilis* × *C. deliciosa*) (Jhalegar et al., 2015). Elevated ethylene levels can stimulate heightened respiration and lead to an increase in weight loss. The clove essential oil exhibited the greatest percentage of weight reduction, which was much higher than that of chlorine and distilled water. Cloves contain eugenol and 3-Allyl-6-methoxyphenol, the major compounds found in high amounts and belong to the phenolic category. The infiltration of hydrocarbon molecules, such as eugenol and chavibetol, into clove essential oil can cause harm to the membranes of cell walls. Hydrocarbon chemicals that possess a high level of toxicity can immediately harm plant cell walls upon contact (Baker, 1970). Excessive amounts of EO might result in burns on the surface of the fruit. The oval sapodilla fruit (*Manilkara zapota* L.) was treated with the maximum concentration of  $500 \mu\text{L L}^{-1}$  EO and a combination of  $500 \mu\text{L L}^{-1}$  EO with 2%  $\text{CaCl}_2$ . This treatment resulted in a decrease in consumer acceptance due to a reduction in fruit quality (Bahmani et al., 2015). Figure 3 illustrates the loss in firmness, decline in total soluble solids, and increase in total titratable acidity of watery rose apple fruit treated with various sanitizing methods. Nevertheless, irrespective of the treatment, all fruit samples exhibited negligible disparities in firmness, total soluble solids, and total titratable acidity levels. Therefore,



applying essential oil washing treatments did not produce any significant effects on these alterations. Fruit firmness can be diminished by various factors, including the transpiration process and cellulolytic microbes. Cellulolytic bacteria secrete enzymes, including polygalacturonase, pectin lyase, and pectate lyase, that are capable of breaking down pectin found in plant tissues (Souza and Souza, 2013). Extended storage duration leads to an increase in the breakdown of protopectin in the middle lamella, resulting in the conversion of protopectin into dissolved pectin. This breakdown is facilitated by enzymes such as polygalacturonase,  $\beta$ -galactosidase, xyloglucanase, endotransglycosylase, and cellulase (Harker and Hallett, 2019). Pectin degradation leads to a decrease in fruit hardness by weakening the connections between fruit cells. Oxygen ( $O_2$ ) facilitates the breakdown of organic compounds in fruit tissue during respiration, producing carbon dioxide ( $CO_2$ ), water, and heat energy. The heat produced during respiration can lead to transpiration by raising the temperature in the fruit tissue. The temperature gradient between the fruit tissue and its surroundings leads to the diffusion of water vapor from the fruit tissue to the environment through the pores of the fruit skin (Krochta et al., 1994). Watery rose apple cv. 'Dalhari' water contains 86.5% of water. Elevated transpiration resulting from increased respiration caused by microbial infections leads to greater water loss, resulting in decreased turgidity and fruit firmness (Bahmani et al., 2015; Kenneth C et al., 2016). Figure 3 illustrates the decrease in total dissolved solids in all treatments over the course of storage. The reduction occurred as a result of the processes of respiration and fermentation. Respiration results from decomposing different organic molecules dissolved in water, including glucose, fructose, and organic acids. This process generates energy in fruit metabolism (Kenneth C et al., 2016). Furthermore, microbes can increase ethylene levels, ultimately leading to heightened plant respiration. As respiration increases, respiration substrates, such as organic acids and simple sugars (glucose, fructose, sucrose), convert into energy. Microorganisms have the ability to metabolize the glucose present in the fruit for their own life (Wang et al., 2021). Nevertheless, in this study, using EO did not result in a significant difference in total soluble solids compared to the treatment with water and chlorine ( $P > 0.05$ ). Similarly, a prior investigation on watery rose apple cv. 'Dalhari', treated with alginate coating and betel EO, likewise found no notable effect on total dissolved solids (Setiawan et al., 2019). The

titratable acidity of 'Dalhari' watery rose apple fruit increased steadily over the 15-d storage period, whereas the pH readings remained essentially constant (Fig. 3). Figure 4 demonstrates that samples treated with cloves essential oil on the final observation day exhibited a slightly but significantly elevated pH ( $6.83 \pm 0.09$ ) compared to the other treatments. Titratable acidity and pH are important food analysis concepts related to acidity. The measure of pH can forecast microbial development, whereas titratable acidity can determine the impact of organic acids on flavor in food (Sadler and Murphy, 2010). The organic acids present in the fruit undergo acid hydrolysis, resulting in their conversion into simpler sugars such as glucose, fructose, and sucrose. This catabolic process lead to an increase the fruit sweetness by reducing sourness as a result of  $H^+$  ion reduction and pH increases (Rehman et al., 2014). Microorganisms present in fruit can also contribute to the rise in overall titratable acidity. Glucose can be converted into several organic acid molecules, such as malic acid, lactic acid, acetyl-CoA, or acetaldehyde, by anaerobic microbes by the process of glycolysis (Kenneth.C et al., 2016). The watery rose apple fruit washing procedure utilizing EO betel exhibits comparable efficacy to chlorine washing, as evidenced by the lack of substantial disparities in weight loss, hardness, total dissolved solids, total titratable acidity, and pH value compared to chlorine. The findings endorse the use of betel essential oil as an eco-friendly and botanical substitute for chlorine-based washing treatments. In recent years, there have been studies undertaken to investigate alternate methods to chlorine cleaning in many industries, mostly because to the environmental and safety hazards associated with chlorine. Chlorine's interaction with organic compounds can lead to the formation of cancer-causing substances, such as trihalomethanes and haloacetic acid (Moustafa et al., 2022). Moreover, the industrial use of chlorine is associated with the creation of significant amounts of wastewater and the corrosion of equipment (FAO/WHO, 2008). Several countries in the United States have prohibited the use of chlorine for organic products due to these concerns (United States Department of Agriculture Agricultural Marketing Service National Organic Program, 2011). Several studies and review papers have examined the potential of essential oils as a plant-based alternative to commercial chemical sanitizers (de Medeiros Barbosa et al., 2016; Esua et al., 2017; Rashid et al., 2020; Gurtler and Garner, 2022; Pizzo et al., 2023). Nevertheless, the precise process by which essential oils reduce

levels of bacteria remains uncertain and cannot be universally characterized, as each essential oil contains distinct antimicrobial components. As previously mentioned in this study, EO comprises a multitude of intricate chemical components. These chemicals possess inherent antibacterial activities, either individually or when combined with other compounds, resulting in amplified bactericidal effects. A study conducted by Ambrosio et al. (2019) found that limonene, which is the primary component of several citrus essential oils, such as lemon, cannot be attributed entirely to the antibacterial capabilities (Ambrosio et al., 2019). Several minor compounds, such as linalool and sabinene, can interact with limonene at the microbial levels to enhance their antimicrobial effect (Angane et al., 2022). Additionally, the utilization of multiple EO (de Medeiros Barbosa et al., 2016) in conjunction with other decontamination techniques as ultrasound (He et al., 2021) has shown an enhanced antimicrobial effect of single-use essential oil. However, there are still other aspects of essential oil usage that require further investigation in order to determine its effectiveness as a substitute for industrial chlorine. An appropriate balance of essential oil content that achieves maximum antibacterial activity without compromising the organoleptic qualities of different fresh produce has not been achieved. Endive leaves were immersed in lavender and spearmint essential oil solutions of varying concentrations for a duration of 5 min. Subsequently, a consumer acceptance test was conducted on these treated leaves (Xylia et al., 2017). The study found that panelists observed alterations in scent perception at elevated concentrations, irrespective of the specific essential oil. While organoleptic testing was not conducted in this study, early laboratory testing did not detect any changes in taste. However, there was a faint remaining scent from the essential oil, potentially as a result of its greater concentration compared to the concentration provided in the present study.

## Conclusions

The study explored the ability of several EOs and chlorine to replace natural substances in the washing process of watery rose apple fruit cv. 'Dalhari'. There was no noticeable variation in the quality of watery rose apple fruit and the amount of surface bacteria when using 0.1% betel essential oil compared to washing the fruit with a 20 ppm chlorine solution. Ultimately, the process of cleaning watery rose apple fruit can be accomplished by utilizing 0.1% betel essential oil

as a plant-derived alternative, which possesses comparable disinfecting qualities to a chlorine wash.

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## Conflict of Interest

The authors indicate no conflict of interest in this work.

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