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Development of Clove (*Syzygium aromaticum*) and Cinnamon (*Cinnamomum burmannii*) Based Food Sanitizer

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Abstract

Although minimally processed food contains more beneficial nutrients, it is one of the largest sources of food-borne diseases. Therefore, this research aims to develop the efficiency of food sanitizer, targeted toward fresh food products using a green chemistry approach. The cinnamon and clove were extracted using water distillation and ethanol extraction with the green technique. The extracts were characterized for antimicrobial activity and incorporated into basic food sanitizer formulation. The solution's color and stability were evaluated and the sanitizer was applied to decontaminate fresh strawberries. The total microbial load before and after the application was also compared to determine the effectiveness of the food sanitizer. Based on the results, all the extracts showed high effectiveness in inhibiting various spoilage microorganisms that exist in food produced with water distillation. The extracts also showed better ability when incorporated into a water-based sanitizer. All the developed food sanitizers can reduce the microbial load of the fresh produce by 4 log per 5 minutes of contact time. Meanwhile, the water-distilled clove extract showed the most effectiveness, decreasing microbial log by 3.93±0.07 log CFU g^{-1} of bacteria load and 4.37±0.14 log CFU g^{-1} of mold load, respectively which performed good dispersion stability for approximately 10 days of observation. This indicated that food sanitizer using water-distilled clove extract could be applied as a good alternative to chemical-based sanitizer.

Keywords: antimicrobial activity; ethanolic extract; food sanitizer; minimally processed food

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INTRODUCTION

The covid pandemic and climate change have increased consumer behavior regarding food consumption. This is due to the increased awareness of consuming food that can contribute to a healthier and sustainable lifestyle. The consumption of fresh or minimally processed fruits and vegetables has been prevalent because of the bioavailability of numerous vitamins, minerals and other phytochemical compounds that are part of the superfood group (Raffo and Paoletti, 2022). However, these processed foods are the leading cause of foodborne illness outbreaks implicating virulent pathogens (Lynch et al., 2009; Olaimat and Holley, 2012; Carstens et al., 2019). According to Sarno et al. (2021), the foodborne disease is a global issue that significantly impacts health. It is mainly caused by the consumption of foods that disease-causing microbes or pathogens have contaminated, which are found in fresh fruits

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and vegetables, including Gram-positive, Gram-negative and fungi (Tournas and Katsoudas, 2005; Dos Santos et al., 2021). Contamination of raw fruits and vegetables with pathogenic organisms may occur during production, harvest, processing, transporting or handling before consumption. Some microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* and *Listeria* may cause illness due to their toxicity (Carstens et al., 2019). These cases have increased awareness regarding the dangers of microbial contamination.

The heating process is usually enough to eliminate the contaminant, but it is not frequently applicable in terms of fresh or sliced food produced. The use of electricity heat treatment to reduce total microbial load is also not efficient in terms of application and cost-effectiveness due to high energy. Other heat treatment alternatives using ozone have been carried out, but the application was limited to the instability of ozone and short life span due to its high oxidative nature (Sarron et al., 2021).

Chemical-based sanitizers often used chemicals that can be harmful to exposed humans or toxic to aquatic species in wastewater. Many surfactants biodegrade slowly into a more toxic, persistent, and can accumulate in the ecosystem. This contributes to the nutrient-loading in water bodies and inhibiting the growth of wild microbiota, therefore, a more sustainable alternative method is required. Fresh food sanitizers are also complicated because they need to be a no-rinse type, which should be applied at an exact concentration with an appropriate contact time to effectively remove contaminants and be safe for human consumption. According to O'Brien (2016), sanitizing effects are usually time-dependent responses, hence, all approved sanitizers will have strict dosing to follow (O'Brien, 2016). This has led to the search for an environmentally friendly washing agent that is harmful and uses a minimal chemical solvent but can eliminate most pathogenic organisms on the surface of fresh produce or minimally processed food (Ahmed et al., 2017; Wang et al., 2020).

Several environmentally safe antimicrobials have been made. Recently, the United States Department of Agriculture (USDA) approved a washed for fresh produce and slices of fruit using a combination of organic acid, fruit acid and hydrogen peroxide (O'Brien, 2016). Another sanitizer peroxyacetic acid, which is a combination of an equal amount of hydrogen peroxide and acetic acid also has been approved for use on fresh produce (Zoellner et al., 2018). Furthermore, chitosan, a polysaccharide, can be used as a good antimicrobial and antiviral on fresh fruit and vegetables (Gutiérrez-Martínez et al., 2018; Valdez et al., 2022). The Food and Drug Administration (FDA) approved niacin antibacterial produce from the microbial fermentation of peptide to be applied in food production (Shin et al., 2016; Berrios-Rodriguez et al., 2019). All of these agents showed the potential to produce an environmentally friendly product with good antimicrobial properties that can be applied to fresh produce.

Plants extracts are widely used in food technology industries because they are majorly considered safer compared to chemical sources (Gutiérrez-Martínez et al., 2018). Chen et al. (2021) stated that a food sanitizer based on the microemulsion of carvacrol, namely a phenolic compound found in oregano could remove 99.9% of the total challenge contaminant. However, the antimicrobial activities of the sanitizer were lost during 14 days of storage (Chen et al., 2021). Other sanitizers using nutmeg oil also showed good stability in concentrated form after several freeze-thaw methods (Agustin and Taihuttu, 2021). In this research, a food sanitizer based on plant essential oil was made using a natural clove and cinnamon extracts. Both extracts have a strong antimicrobial activity due to their phenolic content such as eugenol and cinnamaldehyde, which are active against several pathogenic microorganisms namely *Listeria monocytogenes*, *E. coli*, *Salmonella enterica*, *S. enteritidis*, *Campylobacter jejuni* and *S. aureus* (Ahmad et al., 2005; Sanla-Ead et al., 2012; Tarek et al., 2014). These microorganisms are responsible for food-borne diseases, specifically in fresh or minimally processed food. Clove extract showed superior antimicrobial stability upon storage, which is preferable as an active compound in food sanitizer (Robiatun et al., 2022).

The two extraction methods were carried out to extract different compounds in clove and cinnamon. The extraction methods were designed to reduce waste and use environmentally friendly solvents such as water and ethanol to produce essential oil (the volatile materials that are immiscible with water). Subsequently, the extracts were compared based on their compatibility with the sanitizer and efficacy in

reducing the total microbial load of the fresh produce. The base of the food sanitizer was made with glycerol and propylene glycol which are safe for direct consumption (Kurniawan et al., 2012; Ningsih et al., 2017). The food sanitizer was applied directly to fresh food produce and the initial load of microorganisms was compared with a load of microorganisms after submersion. An effective sanitizer should reduce up to 5 log of microbial load within 30 seconds of contact time (Mohapatra, 2017). In this research, the concentration of the extract was added to achieve the total microbial reduction in the short amount of contact time.

MATERIALS AND METHOD

Materials used were clove buds (*Syzygium aromaticum*), Cinnamon bark (*Cinnamomum burmanii*), distilled water, ethanol, carboxymethyl cellulose (CMC), glycerin, propylene glycol. Cultures of *S. aureus*, *E. coli*, *Aspergillus niger, Rhizopus stolonifer, Rhizopus oryzae* and *Mucor circinelloides* were tested against both water-distilled and ethanol extracts. Meanwhile, the materials used for analysis are media such as nutrient agar (NA) (Merck), plate count agar (PCA) (Merck), potato dextrose agar (PDA) (Merck) and physiological salt solution (Merck). The instrument used for analysis was UV-spectrophotometer 1800 Rayleigh, Colony counter HiMedia LA660 C and Minolta Chromameter CR-400.

Clove and cinnamon extract preparation

The extracts are prepared following the procedures from Amelia et al. (2017) and El Atki et al. (2019). Dried clove and cinnamon bark are obtained commercially and further dried in a 100 ºC oven for 6 hours to reach approximately 10% water content. The dried spices were ground using a blender into powder. Subsequently, the powders were extracted using water distillation and maceration extraction methods to obtain the water-distilled and plant ethanol extracts. For the distillation method, distilled water was used as the solvent with a ratio of 1:10 [w/v] for about 6 hours. In the maceration method, ethanol was used as the solvent to extract both powders at room temperature for 24 hours. The ratio of powder extract to solvent was 1:5 [w/v]. The maceration process was carried out with shaking using a mechanical shaker. The obtained filtrates were concentrated using a rotary evaporator at 45 to 55 ºC with a pressure value of 175 bar. Finally, the extracts obtained were stored in a dark bottle at 4 ºC and analyzed for their yield and minimum inhibitory concentration toward several tested microorganisms.

Preparation of working culture

In this research, the working cultures were prepared following the procedures from Sanla-Ead et al. (2012). The cultures used included *S. aureus, E. coli, A. niger, R. stolonifer, R. oryzae* and *Mucor* sp. Stock cultures were prepared from the pure culture by inoculating one loop of the microorganism inside different test tubes filled with 10 ml of nutrient broth (NB) or potato dextrose broth (PDB) media. The mixture was homogenized using a vortex, passed through 24 hours of incubation at 37 ºC and 48 hours at room temperature for bacteria and mold, respectively. After incubation, 1 loop of each microorganism was streaked to a slant agar and incubated again at 37 ºC and at room temperature for 24 hours and 48 hours, respectively. Subsequently, from the slant agar, 1 loop of the culture was taken and put inside 10 ml of sterile NB or PDB and homogenized using a vortex. The culture stock of bacteria and mold were incubated for 24 hours at 37 ºC inside the incubator and room temperature for 48 hours, respectively.

The working culture was prepared by taking 1 ml of the stock culture and putting it inside a test tube filled with 9 ml of sterile NB or PDB. This was homogenized using a vortex and incubated for 24 hours at 37 ºC for bacteria and 48 hours at room temperature for mold. After incubation, the optical density of the microorganisms was measured at 600 nm using a UV-vis spectrophotometer Rayleigh 1800 before usage. The culture was ready to be used when the OD600 was in the range of 0.3 to 0.4.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC of the extracts were determined using a good diffusion method against *S. aureus, E. coli, A. niger, R. stolonifer, R. oryzae* and *Mucor* sp. based on the procedure from Parhusip and Sitanggang (2011), by measuring the inhibition zone. NA media was used for microorganisms such as *E. coli* and *S. aureus*, while *A. niger, R. stolonifer, R. oryzae* and *Mucor* sp. were grown in PDA that has been acidified by the addition of 1.6% of tartaric acid (10%).

In the well-diffusion method, sterile NA/PDA was inoculated with 0.2% v/v of the culture with an optical density in the range of 0.3 to 0.4 (600 nm) after incubation for 24 hours in NB or PDB. Subsequently, the inoculated media were poured and solidified inside a petri dish. When the agar media solidified, four wells with a diameter of 6 mm were made using sterile micropipette tips and toothpicks. Clove extract or clove oil that has been diluted with sterile dimethyl sulfoxide (DMSO) into four different concentrations was put inside the well. The petri dish was incubated for 24 hours at 37 ºC inside the incubator for bacteria and 48 hours at room temperature for mold. The inhibition zone indicated by a visible area around the well was measured using a digital vernier caliper with a precision of 0.1 mm.

Development of extract-based sanitizer

The extract-based sanitizer was prepared using the procedures by Chen et al. (2021) and Robiatun et al. (2022). The number of extracts added was 5 times the highest minimum fungicidal concentration (MFC) or MBC value, while the food sanitizer based was made according to the formulation listed in Table 1.

Table 1. Formulation of food sanitizer base

Materials	Formula
CMC	2 g
Glycerine	3 g
Propylene Glycol	1.5 g
Distilled water	120 ml

Approximately half a portion of the distilled water was heated until 70 ºC. Subsequently, CMC was added gradually and gently stirred until no lumps were present. Glycerine and propylene glycol were added with the rest of the distilled water. Stirring was carried out until the mixture became a gel. The gel sanitizer was stored in a dark environment at a temperature of 10 to 15 ºC overnight to stabilize the mixture. The clove oil or extract was also added to the sanitizer and homogenized. The gel sanitizers were analyzed regarding their physicochemical properties, namely pH, color, solubility and stability, and total plate count test.

Physicochemical test

The physicochemical test covered the color, pH, solubility and stability of the water-distilled extract-based sanitizer (WDS) and ethanol extract sanitizer (EES) (Ningsih et al., 2017; Robiatun et al., 2022). The color of the sanitizers was measured using a Minolta chromameter and the pH was estimated using pH universal. Solubility of WDS and EES were observed by applying the sample to a microscopic glass. Subsequently, any presence of lumps and coarse particles was observed directly and through a microscope with 1000 to 10000 x magnification. The stability of WDS and EES was observed on days 0, 5 and 10 by observing the separation.

Challenge test

Total plate count (TPC) was carried out as the challenge test of this research on fresh strawberries (*Fragaria anannassa*) (Berrios-Rodriguez et al., 2019; Chen et al., 2021). Strawberries were selected due to their susceptibility toward spoilage and physically sorted for physical defects, namely bruises and cuts as well as contaminants such as dirt and soil. Before application and the TPC test, all the food sanitizers were diluted 5 times with distilled water. Subsequently, the fruits were dipped in a sanitizer solution diluted with distilled water for 300 seconds before putting it inside the dilution bottle. Those from the same batch were also tested without dipping inside the sanitizer solution as the control.

TPC was carried out by putting 50 g of strawberries inside a dilution bottle filled with 200 ml of physiological salt solution. Subsequently, a sample from the dilution bottle was diluted from 10^{-1} to 10^{-4} and used for plating TPC, Duplo. Approximately 1 ml of the diluted sample was added to a petri dish and PCA was used as the media for bacteria, while PDA was for yeast and mold. The petri dish was incubated for 24 hours at 37 ºC and 48 hours at room temperature for PCA and PDA, respectively. The sanitizer produced was considered effective 99.999% or 5 log reduction of the total microorganisms when the sample was in contact with the sanitizer solution for a maximum of 5 minutes (Mohapatra, 2017).

Statistical analysis

All statistical analyses were statistically interpreted using ANOVA with a confidence level of 95% ($\alpha = 0.05$) and further analyzed using Duncan's post hoc test analysis. Complete factorial randomized designs were applied to all experiments with four repetitions. For the extract preparation step, one factor was analyzed, which is the methods of extraction. For the challenge test of the obtained clove sanitizer,

two factors were analyzed, which are the types and dilution of the sanitizers.

RESULTS AND DISCUSSION

Clove and cinnamon extract antimicrobial properties

Table 2 shows the yield (%) of all extracts obtained through distillation and maceration methods. Based on the statistical analysis, there is a significant effect (≤ 0.05) of different extraction methods on yield (%), which indicates a $p < 0.05$ difference between the extraction methods in the yield of the extract.

As shown in Table 2, the distillation method gave a yield of approximately 4.73% for clove with a standard deviation of 0.24. These results are similar to previous research which stated that the average yield of Java clove water-distilled extract obtained from the distillation method ranged from 4.58% to 4.99% (Tarek et al., 2014; Faujdar et al., 2020). The main constituent of clove water-distilled and ethanolic extracts was eugenol, eugenol acetate and β-cariofileno (Amelia et al., 2017). For the cinnamon extract, the yield of maceration extraction was an average of 24.63±0.22 compared to the yield of distillation extraction, which was 0.79±0.05. The yield of water-distilled extract in this research was higher compared to a previous report, where cinnamon extract ranged from 0.48% to 0.50%, and the maceration extraction of *C. burmanii* with 96% ethanol gave an average of 29.25% (Li et al., 2014; Tarek et al., 2014). The main constituent of the cinnamon extract was cinnamaldehyde, while the ethanolic contains phytochemical compounds, including alkaloid, saponin, flavonoid, tannin and polyphenol (Li et al., 2014; Wong et al., 2014; Liu et al., 2017).

The antimicrobial activity of cinnamon-water-distilled and the cinnamon extract was determined using a well-diffusion assay, and the MIC against tested microorganisms is shown in Table 3. According to the statistical analysis, there was a significant effect ($p \leq 0.05$) of the extraction method on the MIC of water-distilled and ethanol extracts against several tested microorganisms.

The antimicrobial activity of both water-distilled extracts was higher compared to the ethanol extract. Similarly, Gupta et al. (2008) reported that the antimicrobial activity of *Cinnamon zeylanicum* water-distilled extract on several foodborne microorganisms was higher than the ethanolic extract. Within tested microorganisms, most mold species were more susceptible to antimicrobial activity compared to the bacteria species (Lixandru et al., 2010). It was discovered that mold was more susceptible to antimicrobial agents followed by Gram-positive and Gram-negative bacteria (Liu et al., 2017). Meanwhile, within the mold species, *R. oryzae* and *M. circinelloides* were the most susceptible, followed by *A. niger* and *R. stolonifer*. Nzeako et al. (2006) stated that clove extracts caused a considerable reduction in the quantity of ergosterol, which is a specific fungal cell membrane component responsible for maintaining cell function and integrity.

Table 2. Effect of extraction methods on yield (%)

Type of extraction	Average yield of clove extract (%)	Average yield of cinnamon extract (%)
Water-distilled extract	$4.73±0.24^a$	$0.79±0.05^a$
Ethanol extract	$23.25±0.53^b$	$24.63±0.22^b$

Note: Different superscripts indicate there is a significant difference (less ≤ 0.05) in the Duncan test after four replications

Table 3. Antimicrobial activity of clove and cinnamon extracts

Tested microorganism	MIC (mg ml^{-1})±SD		MIC (mg ml^{-1})±SD	
	Clove WDS	Clove EES	Cinnamon WDS	Cinnamon EES
E. coli	$0.48±0.15^a$	$0.77±0.18^b$	$0.09±0.02^a$	$0.15±0.02^b$
S. aureus	$0.42±0.22^a$	$0.64±0.22^a$	$0.22±0.07^a$	$0.33±0.01^b$
A. niger	$0.10±0.03^a$	$0.33±0.03^b$	$0.12±0.01^a$	$0.16±0.00^b$
R. stolonifera	$0.14±0.06^a$	$0.22±0.01^b$	$0.21±0.07^a$	$0.34±0.00^b$
R. oryzae	$< 0.10±0.03^a$	$< 0.22±0.01^b$	$< 0.09±0.02^a$	$< 0.15±0.02^b$
M. circinelloides	$< 0.10±0.03^a$	$< 0.22±0.01^b$	$< 0.09±0.02^a$	$< 0.15±0.02^b$

Note: Different superscripts indicate a significant difference (less ≤ 0.05) between WDS and EES of similar origin. SD = standard deviation

Table 4. Physicochemical properties of clove and cinnamon extract sanitizer

Parameters	Clove WDS	Clove EES	Cinnamon WDS	Cinnamon EES
pH	6.4±0.25	5.7±0.45	6.1±0.17	5.4±0.13
Color	55.74^o (yellow-red)	65.67^o (yellow-red)	30.54 (Red)	37.92 (Red)
L* (lightness)	46.25±0.75	39.05±0.63	46.42±1.98	39.31±1.07
Stability (10 days)	Stable	Stable	Stable	Stable
Homogeneity	Dispersible	Not dispersible	Dispersible	Dispersible

Cinnamon is known to cause alterations in the mycelia morphology of mold, where the mycelia will shrivel, and the hyphae collapse (Li et al., 2014). Many reports on the antimicrobial action of the water-distilled and its extract showed disruption of the bacterial and fungal membranes as it compromises the structural and functional integrity of cytoplasmic membranes (Lixandru et al., 2010; Li et al., 2014). *S. aureus* was more susceptible within bacteria compared to *E. coli*, which is a Gram-negative bacteria due to a more complex cell wall than the Gram-positive. Moreover, Gram-negative also have a plasma membrane outside of the peptidoglycan layers, namely the outer membrane. The peptidoglycan of Gram-positive bacteria is relatively porous, which makes most substances pass through the cell wall with less difficulty. The active component of both clove and cinnamon can induce the lysis of bacteria by the leakage of protein and lipid content (Gupta et al., 2008). Therefore, the ethanolic and water-distilled extracts were added to the basic formulation of a food-based sanitizer consisting of glycerol and propylene glycol.

Physicochemical properties of ethanolic and water-distilled extract-based sanitizer

The developed sanitizers were analyzed for their physicochemical characteristic. The EES has lower pH than the WBS. Since ethanol is acidic, the ethanolic extract will have a relatively lower pH than water-distilled, which is generally water-based. The pH values of all sanitizers were 5.4 to 6.5 as presented in Table 4 and can still be considered safe for direct application. For the result of homogeneity analysis, sanitizer combined with water-distilled extract has better homogeneity than the combination with ethanol extract. The water-distilled extract is perfectly dispersed in form of the microemulsion, while the phenolic materials form lumps that are separated from the mixture. The basic formulation of sanitizer contains a CMC, which is a natural emulsifier that helps with the dispersion of all the extract to the polar solvent.

Based on the stability observed on days 0 and 10, there was no separation in all extract sanitizers. This indicates that all sanitizers have possessed homogenous consistency with no separation, although both ethanolic extracts showed a slight color in the EES. The color of the sanitizer also varied based on the original extract, where cinnamon-based extract produced a more reddish color compared to the pale yellow of the clove (Haro-González et al., 2021).

The presence of CMC encapsulated and dispersed the water-distilled extract evenly and oil granules were observed under microscopic view with 1000x magnification. The solid ethanolic extract showed a noticeable separation from the EES. From the imaging in Figure 1, the water-distilled extract showed a good dispersion rate and produced a microstructure that was embedded in the solution. Meanwhile, the ethanolic yields clumps of solids in the mixture, with a coarse dried particle that was difficult to re-dispersed in an aqueous substance. The good dispersion ability of the water-distilled extract might contribute to the antimicrobial activity of the oil-based sanitizer, showing superiority compared to ethanol. According to Haro-González et al. (2021), ethanolic extract usually contains more impurities, waxes and other organic compounds.

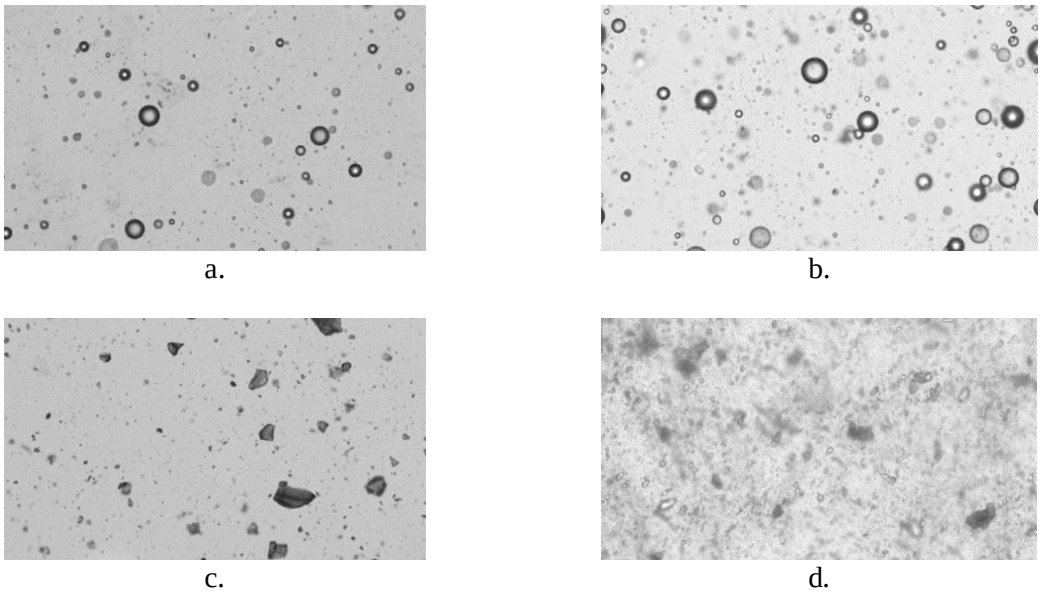
Test of clove and cinnamon based food sanitizer by TPC

Mohapatra (2017) stated that an effective sanitizer could reduce approximately 5 log of microbial load within 30 seconds of contact time. In this research, a challenge test was conducted by performing a TPC analysis to check the microbial count on the surface of the strawberry before and after submerging in the extract with 1, 3, 5 MBC/MFC concentration for 5 minutes with a 1:5 ratio toward the water. The total microorganism of fresh strawberries was used as a control and subtracted from the submerged strawberries with extract, namely a delta log. The statistical analysis showed a significant effect ($p \leq 0.05$) of types of sanitizers and MBC/MIC concentration on bacterial and yeast/mold count

(delta log). From Table 5, a higher decrease in the log value was observed in the strawberries applied with water-distilled extract compared to ethanol. As discussed before, all the water-distilled extracts showed higher antimicrobial activity against both bacteria and fungi than the ethanol extract. After being applied to the food, the extract decreases bacterial and yeast/mold load.

There is a significant effect of less $p \leq 0.05$ on types of sanitizers and concentration of extract toward both bacterial and mold count, namely delta log. A higher decrease in the log value was also observed in the strawberries applied with water-distilled extract-based sanitizer compared to the ethanolic extract-based. Furthermore, both extracts of clove caused approximately a 3.6 to 4.2 log reduction in bacterial and mold count of fresh strawberries. The developed food sanitizer has not reached the standard 5 log of dilution, compare to the use of hydrogen peroxide or acidified sodium chloride sanitizer (O'Brien, 2016; Wang et al., 2020), hence, optimization of the amount of extract and contact time added still need to be conducted.

Clove oil has superior ability in transdermal drug delivery due to its water-soluble terpenoid content (Alghaith et al., 2021), which increases the diffusion coefficient of the drug and explains the efficacy of water-distilled extract compared to ethanol. Lixandru et al. (2010) stated that the clove-based sanitizer could reduce more bacterial and yeast/mold counts by up to 1 log difference. Clove oil is rich in terpenoids and also showed a better diffusion coefficient than cinnamon oil, making it more efficient in reducing the total microbial load (Alghaith et al., 2021). The developed sanitizer showed a similar result to chemical sanitizer. According to Ahmed et al. (2017), the use of non-chlorine sanitizers can reduce the total viable bacteria by 2.3 to 3.5 log CFU g^{-1} and coliform bacteria by 1.1 to 1.9 log CFU g^{-1} on tomatoes, which are lower than the clove (Ahmed et al., 2017; Berrios-Rodriguez et al., 2019). The application of both extracts also showed better reduction compared to another natural sanitizer. This was reported in the research by Wang et al. (2020), where polyhexamethylene guanidine hydrochloride (PHMG) washed lettuce with 150 to 200 mg PHMG concentrated solution (Wang et al., 2020). These showed the potency of using water-distilled extract as a natural sanitizer for fresh food produce.



a. b. c. d.

Figure 1. Microscopic view (1000x) of clove and cinnamon WDS (a, b) and clove and cinnamon EES (c, d)

Table 5. Delta log value of the total microbial count of strawberries (compare to fresh strawberries)

Sample	Δ log CFU g^{-1} bacterial count 5 MBC	Δ log CFU g^{-1} mold count 5 MBC
Clove WDS	3.93 ± 0.21^{de}	4.17 ± 0.14^{e}
Clove EES	3.65 ± 0.17^{d}	3.77 ± 0.15^{d}
Cinnamon WDS	2.12 ± 0.10^{b}	3.19 ± 0.22^{c}
Cinnamon EES	1.74 ± 0.11^{a}	2.02 ± 0.19^{b}

CONCLUSIONS

Water-based extraction of essential oil showed superiority in extracting antimicrobial and antifungal components from both clove and cinnamon extract despite a lower yield compared to the ethanolic extract. In terms of the MBC/MFC (mg ml^{-1}), all extracts yielded significant antimicrobial activity towards a wide range of microorganisms, specifically fungal species. It was discovered that clove water-distilled extract-based sanitizer reduced the bacterial and mold load by approximately 3.93±0.21 and 4.17±0.14 reduction, respectively, with a minimum contact time of 5 minutes which is still below the standard for good sanitizer. Moreover, further research is recommended to optimize extract concentration to increase efficiency and reduce contact time. The stability of the sanitizer upon storage also needs to be observed.

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