



Preparation and Investigation of High-Efficiency Antibacterial Liquid Dishwashing Detergent

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Received: 27 September 2018 / Revised: 1 December 2018 / Accepted: 11 December 2018 / Published online: 20 February 2019
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Abstract

A high-efficiency liquid dishwashing detergent was prepared by using oregano essential oil as an antibacterial agent. The surface cleaning and antibacterial property of the detergent resolved its unfunctionality problem. The antibacterial activities of the detergent were demonstrated through a disk diffusion assay and wipe experiments with *Escherichia coli* and *Staphylococcus aureus*. Results showed that the prepared detergent was highly effective against *E. coli* and *S. aureus*. The results of chemical accelerated tests indicated that the detergent would be effective for at least 1 year. The antibacterial property and detergency performance of the high-efficiency antibacterial liquid dishwashing detergent were compared with those of a commercial antibacterial detergent containing 0.02% *o*-phenylphenol. The detergency performance of the high-efficiency detergent reached 97.8% and was superior to that of the commercial antibacterial detergent.

Keywords Oregano essential oil · Dishwashing detergent · Antibacterial property · Detergency performance

Introduction

Detergents are commonly used in domestic and industrial cleaning. They can be classified as hard surface cleaners, laundry detergents, and dishwashing detergents in accordance with their applications [1]. Although food production technology has been modernized, food safety issues continue to receive increasing attention because of the ubiquity of bacteria, fungi, and other pathogenic microorganisms [2, 3]. Contaminated kitchen surfaces or hands can cause microbial infections because pathogenic microorganisms can adhere to hard surfaces or skin [4]. Surface conditions that promote bacterial growth include water, nutrients, and beneficial temperatures. Bacteria can multiply and form biofilms as microbial communities adhere to one another in extracellular polymeric matrixes [5].

Tableware is prone to contamination and may transfer bacteria to food because it is constantly in contact with the environment, which contains a wide range of bacterial species. Some studies showed that the prevalence of pathogenic bacteria is slightly higher in the homes of users of

non-biocidal products than that in the homes of users of biocidal products but that detergent cleaning can effectively reduce the amount of bacteria on the surfaces of tableware [6–8]. Several types of detergents are commercially available. For example, a commercial antibacterial detergent containing 0.02% *o*-phenylphenol which is classified as irritating to the eyes and skin [9] was used as a comparison in these experiments. Triclosan, another commonly used antimicrobial, may be toxic [10]. In addition, triclosan resistance can develop into cross-resistance to other antimicrobials [11].

It has been found that detergents produced through the fermentation of natural peels have an antibacterial effect [12]. Given the concerns regarding the adverse health effects of synthetic food preservatives, the requirement for antibacterial agents has increased [13]. It was reported that essential oils have bacteriostatic or bactericidal activity against pathogens and can thus be used as natural antibacterial agents [14]. Oregano essential oil (OEO) is volatile oil extracted from oregano. Its main antibacterial ingredient is carvacrol. It is a kind of light yellow oily liquid, which has the advantages of aromaticity, safety, non-poison, high-efficiency sterilization, and no compatibility contraindication [15]. Carvacrol, which is the most active ingredient in phenolic volatile oils and the most important OEO ingredient, can increase cell membrane fluidity and permeability by compressing the

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fatty acid chain of phospholipids; this action causes ions in the cytoplasm to flow out of the cell and results in cell death [16]. Rhoades et al. [17] showed that OEO is an effective antibacterial additive to hand-washing and surface-cleaning detergent solutions and is a potential alternative to triclosan and chloroxylenol.

Mixtures of surfactants are used to improve the application properties of dishwashing liquids and laundry detergents [18]. Environmentally friendly surfactants are often selected to prepare detergents in consideration of economic and environmental factors. The material, application, aging deformation, corrosion resistance, and safety of detergents should be also considered. Cationic surfactants are usually not used in detergents because anions are attached on greasy and soiled surfaces. Anionic surfactants reduce the cleaning power of detergents by precipitating divalent ions from detergents in water with high hardness [19]. Consequently, the addition of zwitterionic and/or nonionic surfactants to anionic surfactants is preferred. Amphoteric surfactants, such as betaines, which possess excellent detergency on oily residues and biodegradability, are used frequently in household detergents [20]. The cleaning of betaines with excellent temperature stability is much better when the nonionic surfactant is used with the amphoteric and can thicken the composition without using a thickener. Consequently, the mixture of cocamidopropyl betaine (CAB-35) and alkyl polyglycoside (APG), with lower skin and eye irritation, was preferred for dishwashing detergents and worked better with oil stain than other detergents.

Although the antibacterial properties of OEO have been extensively studied and exploited, OEO requires further development before it can be considered as a commercial product. The detergency of surfactants toward oily residues has been studied by several research groups [21]. Nevertheless, information on the antibacterial properties of detergents remains limited. Rhoades et al. [17] reported that commercial high-efficiency antibacterial liquid dishwashing detergents containing OEO have been developed. These products contain alkyl ethoxy ether sulfate (AES), alkyl polyglycoside (APG), cocamidopropyl betaine (CAB), sodium hydroxide (NaOH), OEO, and distilled water. In this study, experiments were conducted to characterize the antibacterial activity of an OEO system added to a prepared detergent and to investigate the detergency, antibacterial property, and shelf life of the prepared detergent.

Experimental

Microorganisms and Materials Used

Escherichia coli ATCC6538 and *Staphylococcus aureus* ATCC2592 purchased from China General Microbiological

Culture Collection Center (Beijing, China) were used as test strains. Stock cultures were stored on cryogenic storage beads at $-20\text{ }^{\circ}\text{C}$ and resuscitated through inoculation in 10 mL of nutrient broth medium (10 g L^{-1} peptone, 5 g L^{-1} beef extract, and 5 g L^{-1} NaCl) at $37\text{ }^{\circ}\text{C}$ for 18–24 h when required. Nutrient agar purchased from Beijing AoBoX Biotechnology Co., Ltd (Beijing, China) was used for the subculture of bacteria.

Pharmaceutical grade OEO containing 60% w/v carvacrol and 8% w/v thymol was supplied by Huamei Natural Plant Oil Refinery (Jiangxi Province, China) and used as an antimicrobial ingredient. Sodium hydroxide (NaOH), glycerol monostearate, and distilled water were of analytical grade and purchased from Jiangtian Chemical Industries Co., Ltd (Tianjin, China). The food-grade plant oil was obtained from Handan Chenguang Precious Oil Co., Ltd (Hebei Province, China). Surfactants comprising sodium alcohol ether sulfate (AES, 70 wt%), alkyl polyglycoside (APG, 50 wt%) and cocamidopropyl betaine (CAB, 35 wt%) are one of the most important ingredients in this liquid detergent, which were supplied by Lusen Chemical Industries Co., Ltd (Shandong Province, China).

Preparation of High-Efficiency Antibacterial Liquid Dishwashing Detergent

AES (22 wt%) was slowly dissolved in distilled water (59.2 wt%) at $45\text{ }^{\circ}\text{C}$ and 240 r/min. Then, the APG, CAB, and OEO (8 wt%) mixture was added to the AES solution. The pH of the mixture was adjusted with NaOH (0.8 wt%).

Determination of Detergency

Washing performance was tested with an RHLQ-type multifunctional vertical decontamination-measuring machine equipped with a full set of accessories (China Research Institute of Daily Chemical Industry) to determine the washing performance of detergents containing OEO. Oily residues comprised 5 g of glycerin monostearate and 95 g of plant oil. The oil mixtures were fully dissolved at $80\text{ }^{\circ}\text{C}$. One side of a glass slide was coated with 0.65 g of oily residue and dried for 4 h at room temperature. The stained glass slides were dipped in a wash bucket containing the washing solution, which contained 1.05 g of detergent and 700 mL of hard water. The glass slides were immersed for 1 min at $30\text{ }^{\circ}\text{C}$ and then washed for 3 min at 250 r/min. The efficiency of oil removal (ω) was calculated by using the following formula:

$$\omega = \frac{W_2 - W_3}{W_2 - W_1} \times 100\% \quad (1)$$

where W_1 and W_2 represent the weights of the glass slides before and after the addition of oily residue, respectively,

and W_3 is the weight of the stained glass slides after washing. All of the washing tests were performed three times, and standard error was calculated [22, 23].

Determination of the pH of the Prepared Detergent

pH was measured by a pH transducer (Mettler Toledo Instruments (Shanghai) Co., Ltd) at room temperature.

Determination of Antibacterial Activity

Bacterial Cultures

Staphylococcus aureus and *Escherichia coli* were streaked on nutrient agar plates and incubated at 37 °C for 24 h. A representative colony was removed with a wire loop and transferred to a nutrient agar medium slant, which was then incubated at 37 °C for 24 h. At this stage, the cultures of *S. aureus* and *E. coli* contained $\sim 10^9$ colony-forming units (CFU) per milliliter. Cultures of *S. aureus* and *E. coli* containing $\sim 10^8$, $\sim 10^7$, and $\sim 10^6$ CFU/mL were prepared through dilution with tryptone saline (TPS, tryptone 1 g L⁻¹, sodium chloride 8.5 g L⁻¹). The tubes were then vortex-mixed for 30 s to prepare the bacterial suspensions used for antibacterial tests [24].

Determination of Antibacterial Activity through Paper Disk Diffusion Assay

A sterile cotton swab was dipped in bacterial suspension (10^8 CFU/mL) and used to inoculate the surfaces of 9-cm Petri dishes containing 15 mL of nutrient agar. Next, 20 μ L (2×10 μ L) of the test solution was pipetted on sterile qualitative filter paper disks (5 mm in diameter). The disks were allowed to dry in an open sterile Petri dish on a clean bench (Shanghai Boxun Industry & Commerce Co., Ltd, China) and placed on inoculated agar. The plates were incubated at 37 °C for 18 h. Antibacterial activity was determined by measuring the inhibition zone diameter (IZD, in mm) produced by the solution against microorganisms. The commercial antibacterial detergent containing 0.02% *o*-phenylphenol and sterile distilled water were used as the positive control and negative control, respectively [25].

Determination of Bacterial Count for the Prepared Detergent

Further decimal dilutions were prepared with sterile saline solution as follows: 0, 1/10, and 1/100. Next, 1 mL of diluted samples (positive control for sterile saline) was uniformly smeared onto plates. Nutrient agar was poured into plates containing the samples through the pour plate technique. Plates were incubated at 37 °C for 24 h prior to colony enumeration.

All experiments were conducted three times. Colonies were not observed in the positive control group.

Wipe Experiments

Wipes

Household cleaning cloths were cut into 6 cm \times 6 cm squares and sterilized through autoclaving at 121 °C for 20 min in a sealed container. Each cloth square was placed in a sterile vitreous Petri dish and moistened with 2.5 mL of the test solution. The solution was poured over the surface of the cloth. It was then allowed to disperse within the cloth for a few seconds. The wipes were specifically treated with 2.5 mL of the test solution so that they will be wet to the touch but will not drip if lifted.

Bacterial Inactivation on Wipes

The test solutions used to wet the cloth were diluted with distilled water to 1/16, 1/10, 1/8, and 1/5 (w/w). That is, the concentrations of OEO were 0.5 wt%, 0.8 wt%, 1.0 wt%, and 1.6 wt%. For each test, 100 μ L of bacterial suspension was inoculated in a diagonal line across the square piece of wipe. After exactly 2 min, the wipe was transferred to 50 mL of TPS in a 100-mL beaker and placed in a 85-1 type A magnetic stirrer (Gongyi Yuhua Instrument Co, Ltd, China) for 30 s. Serial decimal dilutions were carried out in TPS as required, and the bacterial count in the diluted sample was determined with the method mentioned above. Raw counts were converted to lg CFU per wipe. An arbitrary value of 2.4 lg CFU per wipe was assigned to cases where no colonies were isolated ($0.5 \times$ the arithmetic assay detection limit) [17].

Determination of Valid Time for the Prepared Detergent

Samples were sealed and placed in a constant temperature and humidity incubator at 54 °C for 14 days. The appearance of the sample was observed. The paper disk diffusion assay (described in section “Determination of Antibacterial Activity through the Paper Disk Diffusion Assay”) was carried out before and after placement. Evaluation standards showed that the decline rate of sterilization was less than or equal to 10% after 14 days at 54 °C and the sample can be effective for 1 year. The decline rate of sterilization β was calculated on the basis of the IZD before and after placement and is expressed by the following equation:

$$\beta = \frac{X_0 - X_t}{X_0} \times 100\% \quad (2)$$

where X_0 and X_t represent the IZD before and after placement, respectively.

Results and Discussion

Detergency Performance Analysis of Oily Soil from Glasses

Table 1 shows five kinds of dishwashing detergent formulations. Figure 1 presents the effects of five kinds of dishwashing detergent formulations at different temperatures.

As shown in Fig. 1a, each prepared detergent at ambient temperature was clear, transparent and faint yellow. The

detergent samples were then placed in a $-5\text{ }^{\circ}\text{C}$ environment, removed after 24 h, and allowed to recover to room temperature. Crystallization and precipitation were not observed (Fig. 1b, c). When placed in the constant temperature and humidity incubator of $(40 \pm 1)\text{ }^{\circ}\text{C}$ for 24 h, removed and observed immediately, the samples had neither delamination nor turbidity (Fig. 1d). This suggests that the products had a good stability.

The six different detergent formulations were subjected to the washing performance test in water with a hardness of 250 ppm and showed excellent washing performance. The results are presented in Table 2. Each formulation achieved oil removal rates of more than 90%, and the relative average deviation of all determined results was less than 5%.

Table 1 Dishwashing antibacterial detergent formulations

Formulations	AES (wt%)	APG (wt%)	CAB (wt%)	NaOH (wt%)	OEO (wt%)	Distilled water (wt%)
OD-1	22	9	1	0.8	8	59.2
OD-2	22	8	2	0.8	8	59.2
OD-3	22	7	3	0.8	8	59.2
OD-4	22	6	4	0.8	8	59.2
OD-5	22	5	5	0.8	8	59.2

OD OEO detergent

Fig. 1 Appearance of five kinds of dishwashing detergent formulations at different temperatures: **a** ambient temperature, **b** $-5\text{ }^{\circ}\text{C}$, **c** room temperature, **d** $40\text{ }^{\circ}\text{C}$

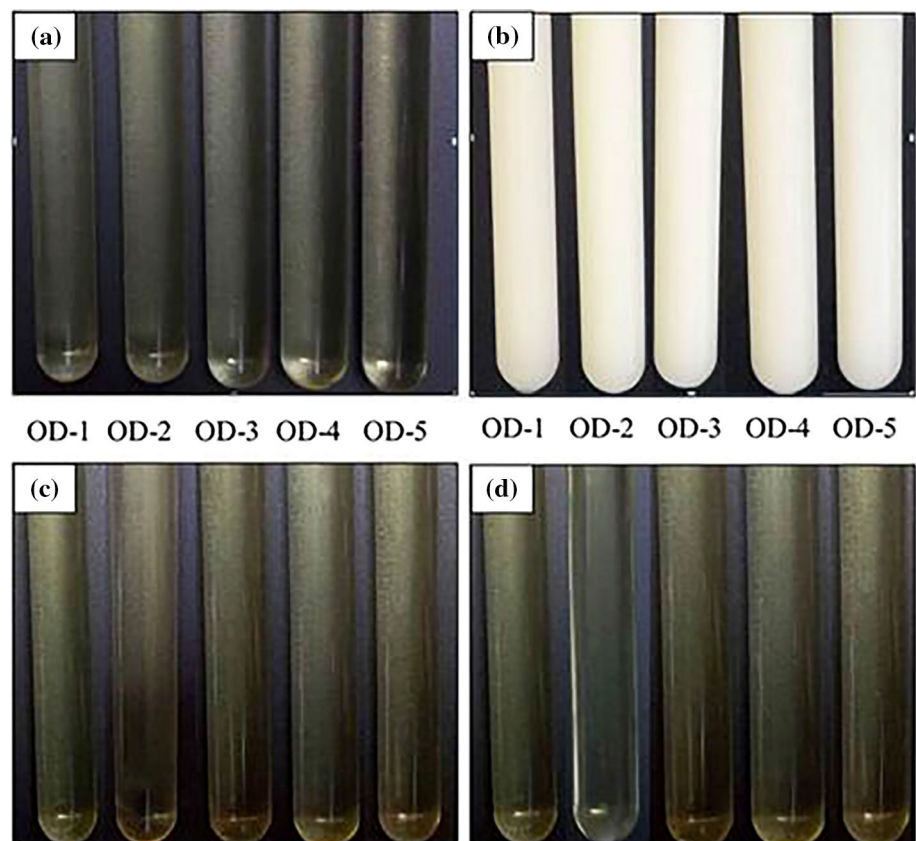


Table 2 Detergency performances of six different detergent formulations

Sample	Oil removal rate (%)			Average value \pm SD (%)	Relative average deviation (%)
OD-1 (8%)	96.7	96.3	96.4	96.5 \pm 0.21	0.2
OD-2 (8%)	97.1	97.1	98.5	97.6 \pm 0.81	0.6
OD-3 (8%)	95.1	96.8	96.4	96.1 \pm 0.88	0.7
OD-4 (8%)	96.0	94.5	93.6	94.7 \pm 1.21	0.9
OD-5 (8%)	85.5	82.1	86.3	84.6 \pm 3.15	2.0
CD (0.02%)	96.3	96.2	96.7	96.4 \pm 0.26	0.2

Values are mean \pm SD of triplicate analysis; CD (0.02%) represents the commercial antibacterial detergent containing 0.02% *o*-phenylphenol; 8% is the concentration of OEO in the OD formulation

The oil removal rate of CD (0.02%) was 96.4%. Among OD-1–OD-5, OD-2 exhibited the highest detergency performance and was thus compared with the commercial antibacterial detergent. Comparative analysis revealed that the decontamination effect of OD-2 reached 97.6% and was superior to that of CD (0.02%). OD-2 was selected for the subsequent experiments on the basis of its oil removal performance.

pH of the Prepared Detergent

The pH of the 1/100 dilution of OD-2 was 10.42, which met the pH requirement of 4.0–10.5.

Antibacterial Activity Analysis for Different Concentrations of OEO

Escherichia coli and *Staphylococcus aureus* were used in the antibacterial activity experiment because they represent the most common nosocomial Gram-positive and Gram-negative bacterial pathogens, respectively [26, 27]. The results for the antibacterial activity of OD-2 and CD are summarized in Table 3. Table 3 shows that OD-2 and CD exhibit broad-spectrum bactericidal performance. IZD values increased as OEO increased. IZD values that exceeded 7 mm were indicative of bacteriostatic action. The IZD values of *E. coli* and *S. aureus* were 6.25 and 10.38 mm, respectively, when exposed to detergent containing 0.5% OEO. Accordingly, less than 0.5% OEO was not bacteriostatic action for *E. coli* and less than 0.25% OEO was not bacteriostatic action for *S. aureus*. Moreover, the IZD values of *E. coli* were less than those of *S. aureus* under each OEO concentration (wt%). That is, *E. coli* was more resistant than *S. aureus*. Control treatment (sterile distilled water) failed to inhibit the growth of any bacteria.

CD (0.02%) exhibited modest inhibitory effects on the tested microorganisms. By contrast, OD-2 (8%) possessed

Table 3 Antibacterial activity of OD-2 (8%) diluted by different multiples and CD (0.02%)

OEO concentration (wt%)	IZD (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
0.125	5.00	5.00
0.25	5.00	5.00
0.5	6.25 \pm 0.07	10.38 \pm 0.08
0.8	7.97 \pm 0.12	16.67 \pm 0.10
1	8.24 \pm 0.17	16.85 \pm 0.40
1.6	8.99 \pm 0.05	17.14 \pm 0.26
2	9.60 \pm 0.15	18.82 \pm 0.30
4	10.72 \pm 0.28	21.22 \pm 0.20
6	12.33 \pm 0.19	22.39 \pm 0.34
8	16.51 \pm 0.04	27.61 \pm 0.22
CD (0.02)	14.61 \pm 0.16	15.17 \pm 0.27

Values are mean \pm SD of triplicate analysis; 5 mm is the size of disks IZD the diameter of inhibition zone

strong antibacterial activity, especially against *S. aureus*. Specifically, the IZD of *S. aureus* was 27.61 mm under OD-2 (8%). Compared with CD (0.02%), which measured at 15.17 mm for *S. aureus*, the IZD of OD-2 (8%) diluted 1/10 with distilled water (namely with the addition of 0.8% OEO) was 16.67 mm for *S. aureus*. Accordingly, this formulation was highly effective against the tested bacteria while being safe for the human body.

The IZD values of 1% and 2% OEO concentration exceeded 25 mm except *A. hydrophila*, *B. cereus* and *E. coli* in the survey of Baydar et al. [28]. As the main antibacterial composition in OEO, 86.95% carvacrol was investigated by Baydar et al. In the present study, a series of experiments were carried out on the OEO containing 60% carvacrol. High OEO concentration may account for the strong antibacterial activity of the detergents tested by Baydar et al.

Analysis of Direct Inoculation for Antibacterial Wipes

Figure 2 shows the effect of different OEO concentrations on bacterial survival. Wipes were soaked with water (0.0%) or detergent solution containing different concentrations of OEO diluted with distilled water as follows: 1/16, 1/10, 1/8, and 1/5 (w/w). In the experiments conducted with the bacterial counts of $\sim 10^7$ CFU/mL (Fig. 2a), the count of *E. coli* was decreased by nearly 1.5 lg CFU per wipe, whereas that of *S. aureus* was decreased by approximately 4 lg CFU per wipe. Bacterial count on the wipe with 0.8% OEO was undetectable. *E. coli* was more resistant and merely reduced by nearly 2.6 and 3 lg CFU per wipe at 0.8% and 1.0% OEO, respectively. No pathogens were observed under treatment with 1.6% OEO.

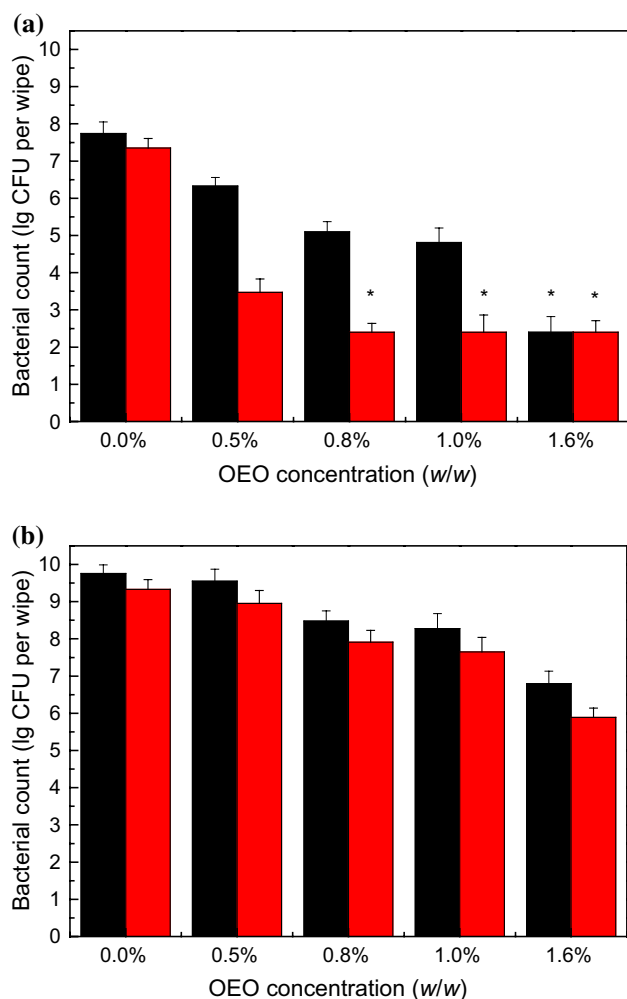


Fig. 2 Bacterial survival rates after 2 min after inoculation onto a wipe soaked with detergent solution containing different concentrations of OEO (bacterial counts of **a** $\sim 10^7$ CFU/mL and **b** $\sim 10^9$ CFU/mL). Bars represent the mean (of three) counts of *E. coli* (red) and *S. aureus* (black). *The bacterial counts of some or all of the three replicates were below the detection limit of 2.7 lg CFU per wipe and were assigned an arbitrary value of 2.4 lg CFU per wipe

The results of the present study differed from those of the study by Rhoades et al. [17]. Rhoades et al. reported that *S. aureus* and *E. coli* were undetectable under treatment with 0.05% and 1% (v/v) OEO. It could be speculated other than the concentration difference of carvacrol—the main antibacterial composition in OEO, soap formulations or surfactants may have different impacts on improving the antibacterial properties of OEO. Moreover, the method used to express OEO concentration in this study differed from that used in the study of Rhoades et al. Specifically, in this study, OEO content was expressed in mass percent, whereas that in the work of Rhoades et al. was expressed in volume percent.

The bacterial inactivation of $\sim 10^9$ CFU/mL from wipes is presented in Fig. 2b. Viable *E. coli* remaining on the

wipes moistened with water at 0.0%, 0.5%, 0.8%, 1.0%, and 1.6% OEO were 9.8, 9.5, 8.5, 8.3, and 6.8 lg CFU per wipe, respectively, while *S. aureus* remaining on the wipes were 9.3, 8.9, 7.9, 7.6, and 5.9 lg CFU per wipe, respectively. *E. coli* was reduced by 0.3, 1.3, 1.5, and 3.0 lg CFU per wipe, while *S. aureus* was decreased by 0.4, 1.4, 1.7, and 3.4 lg CFU per wipe at 0.5%, 0.8%, 1.0%, and 1.6% OEO (relative to the water control), respectively. Figure 2a shows the bar graph of the bacterial counts of $\sim 10^7$ CFU/mL, displaying a marked differences between *E. coli* and *S. aureus* remaining on the wipes. Figure 2b shows the bar graph of the bacterial counts of $\sim 10^9$ CFU/mL, showing no significant differences, but each OEO concentration (wt%) still exhibited *S. aureus* was less resistant than *E. coli*.

Bacterial Count Analysis for the Prepared Detergent

Bacterial count under treatment with OD-2 is given in Table 4. The number of bacterial colonies of antibacterial/bacteriostatic detergent was required to be less than 200 CFU/mL. The mean number of bacterial colonies for the sample OD-2 was 0 CFU/mL at different dilution ratios. No colonies were observed in the positive control group. Results indicated the detergent reached standard requirements.

Shelf life Analysis of the Prepared Detergent

The valid time for the prepared detergent was determined by the microbiological assay which was the effect of killing bacteria for the sample OD-2 before and after placement.

The bacteriostatic test results of OD-2 are given in Table 5. After 14 days, the inhibitory effects of OD-2 on *E. coli* and *S. aureus* were weakened by less than 10%. As the decline rates of killing effect for *E. coli* and *S. aureus* were

Table 4 Microbial counts under treatment with the prepared detergent

Dilution ratio of OD-2	Mean number of bacterial colonies
0	0
10	0
100	0

Table 5 Antibacterial activities of the prepared detergent before and after placement

Storage time (days)	IZD \pm SD (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
0	16.51 \pm 0.12	27.61 \pm 0.16
14	15.01 \pm 0.19	25.79 \pm 0.20

Values are mean \pm SD of triplicate analysis

9.07% and 6.63%, respectively, germicidal efficacy would be valid for at least 1 year.

The stability test results of OD-2 are also presented in Table 5. These results show that the detergents are highly stable. Furthermore, OD-2 was more effective against Gram-positive bacteria than Gram-negative bacteria before and after 14 days of incubation under certain conditions.

Conclusions

A high-efficiency antibacterial liquid dishwashing detergent that resolved the unfunctionality problem of commercially available detergents was prepared in this work. The detergency performance, antibacterial activity, bacterial count of the sample, and shelf life of the prepared detergent were determined. The detergency performance of OD-2 reached 97.8% and was superior to that of a commercial antibacterial detergent, which had a detergency performance of 96.4%. They showed antibacterial activities against *S. aureus* and *E. coli*, but the prepared detergent was more effective against tested bacteria and safer for the human body. The high-efficiency antibacterial property of the prepared detergent is illustrated in Fig. 2, which clearly indicates the differences between the sensitivities of *S. aureus* and *E. coli* to the detergent. The detergents continued to exert antibacterial activity even when bacterial count reached more than 9 lg CFU per wipe. Samples treated with different dilution ratios of OD-2 had bacterial counts of 0 CFU/mL. Chemical accelerated tests revealed the detergents would retain their antibacterial activities for at least 1 year. The results suggest that the OD-2 detergent is more effective against Gram-positive bacteria over a year.

Given that the components of OD-2 are environmentally friendly, non-toxic, and inexpensive, it could be used as an alternative for commercial detergents. This work was mainly performed using laboratory control strains. Further experiments are required to investigate the antifungal activity and surface-cleaning performance of OD-2.

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