J. T. K. L.
JURNAL TEKNIK KIMIA DAN LINGKUNGAN

J. Tek. Kim. Ling. 2023, 7 (2), 114-122
p-ISSN : 2579-8537, e-ISSN : 2579-9746
http://jurnal.polinema.ac.id/index.php/jtkl
DOI: http://dx.doi.org/10.33795/jtkl.v7i2.3737

Potential of Citronella Oil and Gum Rosin as Antimicrobial Agents in Floor Cleaner Liquid Against *Salmonella thypi*

Wa Ode Cakra Nirwana[1,2,*], Chandrawati Cahyani[1,2], Vivi Nurhadianty[1,2]

[1]Department of Chemical Engineering, Faculty of Engineering, University of Brawijaya, MT. Haryono No. 167, Malang, 65145, Indonesia
[2]Institute of Essential Oil, University of Brawijaya, Gedung Senat 1st floor, Veteran, Malang 65145, Indonesia

ABSTRACT

Some floor cleaner products on the market contain antimicrobial agents that are harmful to humans and the environment, such as benzalkonium chloride. Therefore, searching for a new antimicrobial agent that is relatively safe is a significant challenge. In this study, we investigated the best formula for making floor cleaner using citronella oil and gum rosin as antimicrobial agents. Its antimicrobial activity against *Salmonella thypi* was investigated and compared with that of a commercial floor cleaner containing benzalkonium chloride. Five concentrations and ratios of citronella oil and gum rosin were investigated. The quality of floor cleaner in terms of pH range and the stability of the emulsion in hard water was assessed according to SNI 06-1842-1995. The results showed that all formulas met the pH range required by SNI, except for formulas containing citronella oil alone. The emulsion in hard water was stable for all formulas. The antimicrobial activity increased with increasing concentrations of antimicrobial agents. Citronella oil exhibited higher antimicrobial activity than gum rosin. Benzalkonium chloride showed higher microbial activity than gum rosin but lower than citronella oil. The combination of citronella oil and gum rosin showed higher microbial activity by 1.1–2.4 times than formulas containing benzalkonium chloride. The optimum floor cleaner formula (a concentration of citronella oil and gum rosin of 2% with a ratio of 1:1) has an inhibition zone diameter of 22.2 mm.

***Keywords*:** antimicrobial, citronella oil, floor cleaner, gum rosin.

1. INTRODUCTION

Currently, many floor cleaning products offer various advantages such as being able to clean floors, make floors shinier, smell fresh and last longer, and as antimicrobial. Of the many advantages offered, the ability to act as an antimicrobial is the main attraction for consumers. However, some of the antimicrobial agents used are hazardous to humans and the environment.

In Indonesia, floor cleaning products generally use benzalkonium chloride, cresylic acid, ethoxylated alcohol, and pine oil as antimicrobial agents (personal observation). Benzalkonium chloride is dangerous because it is corrosive, causes severe skin burns and eye damage, may cause respiratory irritation, and is very toxic to aquatic life with long-lasting effects [1]. Cresylic acid is toxic, corrosive, cause severe burns if in contact with skin, and can affect the central nervous system and other organs (lungs, liver, kidneys, and eyes) [2]. Ethoxylated alcohol is corrosive, harmful if swallowed and in contact with skin and eyes, and very toxic to aquatic life [3]. Meanwhile, pine oil is relatively safer and does not have the potential to cause chronic health problems, but it can cause irritation if in contact with the eyes and sensitive skin [4].

As previously explained, with the negative effects of antimicrobial agents used in commercial floor cleaning products on human health and the environment, using natural ingredients that are relatively safer in

[*]Corresponding author: Wa Ode Cakra Nirwana
Department of Chemical Engineering, University of Brawijaya
MT. Haryono No. 167, Malang, 65145, Indonesia
E-mail: cnirwana@ub.ac.id

Received : August 6, 2023
Accepted : October 19, 2023

household products, particularly floor cleaners, is highly important. Natural active ingredients that have the potential to be used as antimicrobials in floor cleaners liquid are citronella oil and gum rosin.

The main components of citronella oil are citronellal, geraniol and citronellol [5]. These components have antibacterial properties that can inhibit the growth of bacteria that cause food spoilage and diseases such as *Brochothrix thermosphacta*, *Escherichia coli*, *Listeria innocua*, *Listeria monocytogenes*, *Pseudomonas putida*, *Salmonella typhimurium*, and *Shewanella putrefaciens* [6–9].

Gum rosin is obtained from the distillation residue of the sap of pine trees [10]. Its constituent components vary, strongly influenced by the species of pine, environment, plant age, geographic origin, resin-tapping *techniques*, etc. [11–13]. The main components of gum rosin are diphenic acid, mainly abietic acid, isopimaric acid, laevoabietat acid, and pimaric acid [10]. Several studies have shown that abietic acid has a function as an antibacterial that causes disease [14,15].

To date, research regarding the production of floor cleaner liquid using natural ingredients as antimicrobial agents has been widely explored. The antimicrobial agents used include lerak fruit [16], mangosteen peel [17], kaffir lime leaf oil, lemongrass oil, and its hydrosol [18]. To the best of the authors' knowledge, study of the use of citronella oil and gum rosin as antimicrobial agents in a floor cleaner product has not been investigated. In this work, we investigated the optimum formula of citronella oil and gum rosin as antimicrobial agents in floor cleaner liquid against *Salmonella thypi*. Its antimicrobial activity was then compared with that of a commercial floor cleaner that uses benzalkonium chloride as the antimicrobial agent.

2. MATERIALS AND METHODS

2.1. MATERIALS

Citronella oil was obtained from Essential Oil Institute, University of Brawijaya, Malang, Indonesia. Sodium hydroxide, gum rosin, (MES), nutrient agar, and nutrient broth were purchased from local suppliers.

2.2. PRODUCTION OF FLOOR CLEANER LIQUID USING CITRONELLA OIL AND GUM ROSIN AS ANTIMICROBIAL AGENTS

Five concentrations of antimicrobial agent i.e. 1–5% w/v (mass of citronella oil and gum rosin per volume of aquadest) were evaluated. While the ratio of citronella oil to gum rosin was 1 : 0; 0 : 1; 1 : 1; 2 : 1; 1 : 2 (w/w). The experiments were carried out using a completely randomized design method; hence, the number of formulas was 25.

The flowchart for making floor cleaner liquid is shown in Figure 1. NaOH (0.2 mass of gum rosin) was dissolved in aquadest. Subsequently, gum rosin and the remaining aquadest were added, stirred, and heated at 75 ^{o}C for 15 min. After cooling down to room temperature, MES (0.3 mass of gum rosin) was added. After it dissolves completely, citronella oil was added and stirred until homogenous.

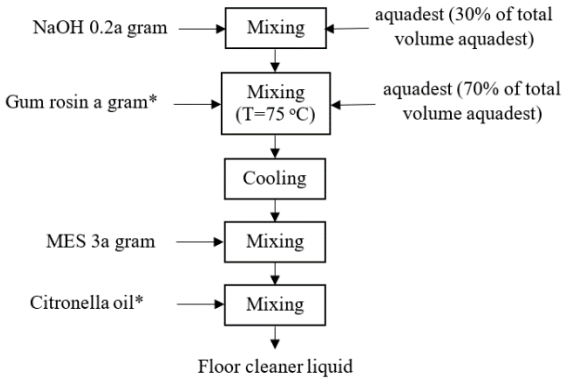
2.3. MICROORGANISM AND INOCULUM PREPARATION

Salmonella thypi used for antimicrobial activity testing was purchased from the Faculty of Medicine, University of Brawijaya. The strain was grown on nutrient agar and incubated at 37 ^{o}C overnight. The inoculum preparation procedure refers to the standard procedure by the Clinical and Laboratory Standards Institute (CLSI) [19] with a slight modification. Four colonies were transferred to 10 mL of nutrient broth and then incubated at 37 ^{o}C for 12 h. The turbidity of the cell suspension was adjusted with sterile nutrient broth to obtain turbidity comparable to the 0.5 McFarland standard

(containing approximately 1.5 x 10^8 CFU/mL).

2.4. ASSESSMENT OF THE QUALITY OF FLOOR CLEANER

The quality of the floor cleaner in terms of pH and stability of emulsion in hard water was assessed according to SNI 06-1842-1995 [20]. The pH was measured periodically for 11 days using a pH meter (OHAUS, Starter 300, USA). The assessment of the stability of the emulsion in hard water was carried out by dissolving the floor cleaner in hard water with ratios of 5:100 and 1:100 (v/v). Subsequently, the stability of emulsion was observed visually after 6 hours. Stability refers to the absence of precipitation or separate layers. For floor cleaners containing phenol and its derivatives, the emulsion in hard water must be stable while for other compounds, it does not form an emulsion (SNI 06-1842-1995).



*The composition of gum rosin and citronella oil was adjusted according to the variables investigated in this work.

Figure 1. Procedures for making floor cleaner liquid with antimicrobial compounds of citronella oil and gum rosin.

2.5. ANTIMICROBIAL ACTIVITY TESTING

The protocol of antimicrobial activity testing using the disk diffusion method refers to CLSI standards with slight modification [19]. A 0.1 mL of bacterial suspension was spread on the surface of a nutrient agar using a bacterial cell spreader. The disks were immersed in the floor cleaner liquid then placed on the inoculated agar surface and incubated at 37 °C. The antibacterial activity was evaluated by measuring the diameter of inhibition zone of the tested bacteria using a ruler as depicted in Figure 2, then recorded as an average, which was expressed in millimeters. As a comparison, the antimicrobial activity of a commercial floor cleaner liquid containing benzalkonium chloride was also conducted to compare their effectiveness against the strain. The concentration of benzalkonium chloride in the floor cleaner liquid was adjusted to the concentrations of the antimicrobial agent specified in this work. The experiments were conducted in triplicate.

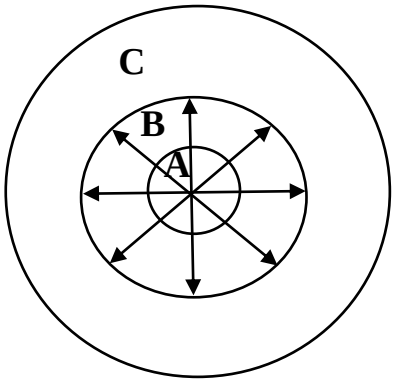


Figure 2. Method of inhibition zone measurement.
A=disc paper; B= inhibition zone; C=petri dish

3. RESULTS AND DISCUSSION

3.1. pH TESTING

According to the Indonesian standard for floor cleaner products (SNI-06-1842-1995), the pH must be in the range of 6–11. As depicted in Figure 3, the pH of floor cleaners varied. The pH was less than 6 when the floor cleaner contained citronella oil alone as the antimicrobial agent. In contrast, using gum rosin resulted in a pH of around 8–10. A combination of citronella oil and gum rosin with various concentrations and ratios produced floor cleaners with a pH of 7–10. Observation of pH changes for 11 days showed that the pH of floor cleaners was relatively stable. As a comparison, the pH of the commercial floor cleaner

containing benzalkonium chloride was 10 (data not shown). These results show that the floor cleaners produced in this work met the pH standards required by SNI, except for formulas that used citronella oil alone. This is because the pH of citronella oil used in this study was 5.

3.2. TESTING OF EMULSION STABILITY IN HARD WATER

The purpose of this test is to observe the stability of floor cleaner emulsion in hard water. Two ratios of floor cleaner to hard water were observed, namely 1:100 and 5:100. Table 1 shows the visualization of floor cleaners in hard water after 6 h with a total concentration of citronella oil and gum rosin of 5% (other concentrations are not shown for simplification). The results show that samples not containing gum rosin did not form emulsions. As previously described, according to SNI 06-1842-1995, for floor cleaners containing phenol and its derivatives, the emulsion in hard water must be stable, while for other compounds, it does not form an emulsion. The main components of gum rosins are abietic acid, isopimaric acid, laevoabietat acid, and pimaric acid which are categorized as phenol derivative compounds, thereby forming an emulsion in hard water. In contrast, the main components of citronella oil are citronellal, geraniol, and citronellol which are categorized as non-phenol compounds. Therefore, it did not form an emulsion in hard water.

After 6 h of observation, all samples showed there was no precipitation, floc formation, or separated layer. It indicates that the emulsion of floor cleaners containing citronella oil and gum rosin is stable and homogeneous in hard water.

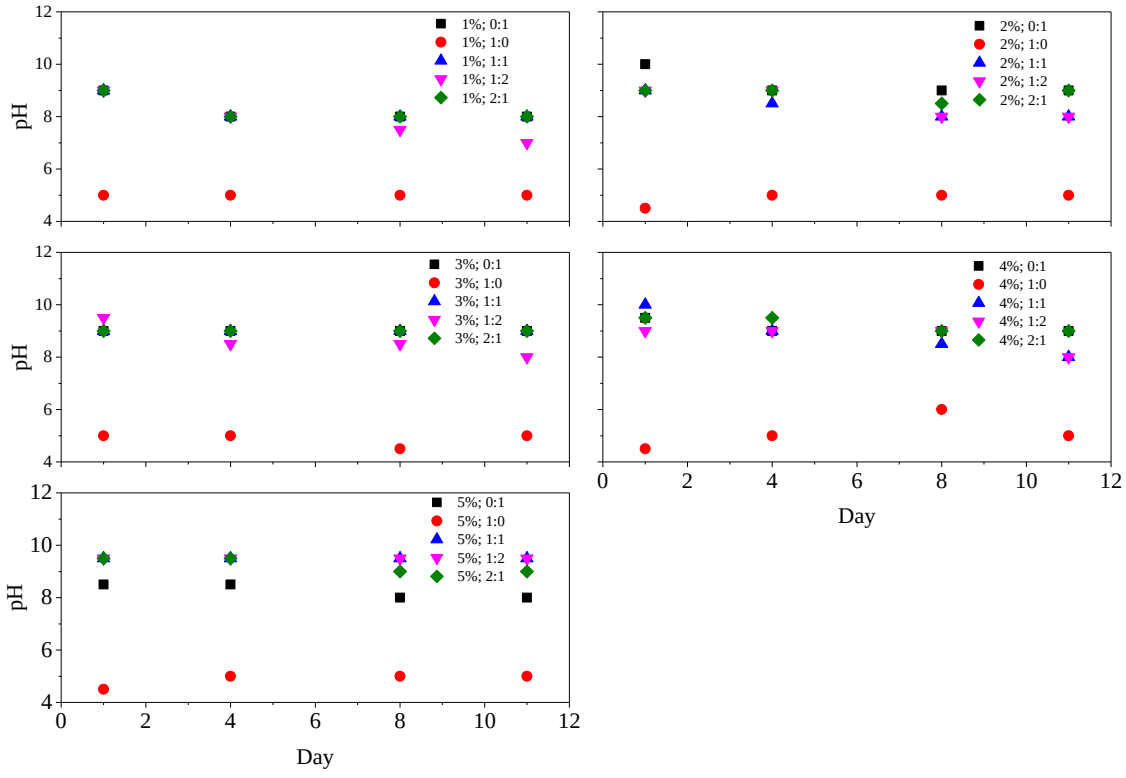
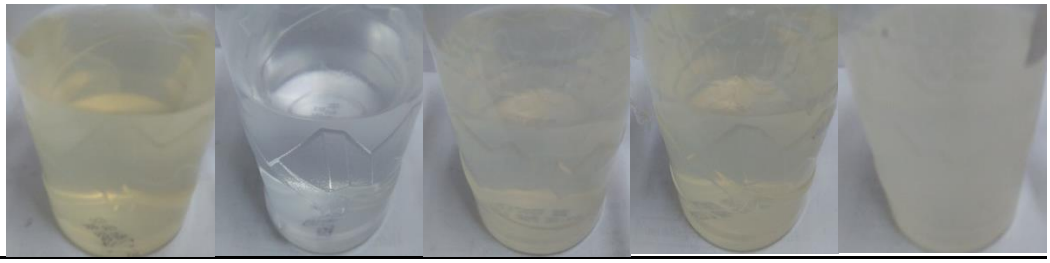


Figure 3. Profile of pH changes in floor cleaners containing citronella oil and gum rosin. Legend represents the total concentration of citronella oil and gum rosin in floor cleaner; ratio of citronella oil to gum rosin (w/w).

Table 1. Visualization of homogeneity of floor cleaners in hard water after 6 h with a total concentration of citronella oil and gum rosin of 5%.

Ratio of floor cleaner to hard water	Ratio of citronella oil to gum rosin				
	0:1	1:0	1:1	1:2	2:1
1:100					
5:100					

3.3. ANTIMICROBIAL ACTIVITY TESTING

Salmonella thypi was chosen as the tested bacteria because this is one of the bacteria that is commonly found on the floor. The antimicrobial activity of citronella oil and gum rosin was assessed according to the size of the clear zone (inhibition zone) around the disc paper. A larger zone of inhibition around an antimicrobial-containing disk indicates that the bacteria are more sensitive to the antimicrobial in the disk and vice versa (Figure 4).

Figure 5 shows the effect of concentrations and ratios of citronella oil and gum rosin in floor cleaner on the inhibition zone against S. thypi. In general, the diameter of the inhibition zone increased with increasing concentrations of antimicrobial agents. Floor cleaners containing citronella oil alone showed a larger inhibition zone of around 2.5–3.1 times than floor cleaners containing gum rosin alone under the range concentrations observed in this work. It indicates that S. thypi is more sensitive to citronella oil than gum rosin. The combination of citronella oil and gum rosin as antimicrobial agents increased the inhibition of S. *thypi* growth. A higher ratio of citronella oil increased the inhibition zone. The same phenomenon was also observed for gum rosin. The highest inhibition zone for each ratio of citronella oil : gum rosin was 0:1 = 7.3 mm; 1:0 = 21.1 mm; 1:1 = 22.2 mm; 1:2 = 22.8 mm; and 2:1 = 19.3 mm, respectively, achieved at concentrations of 3%, 2%, 2%, 5%, and 2%. Citronella oil is well known to have good antimicrobial efficacy since it contains citronellal, geraniol, and citronellol which have been described as antiseptic, antibacterial, and antifungal.

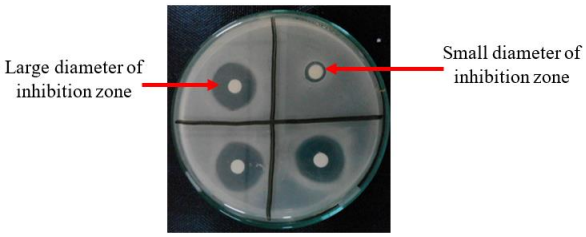


Figure 4. Clear (inhibition) zone around the disc paper on the antimicrobial activity testing.

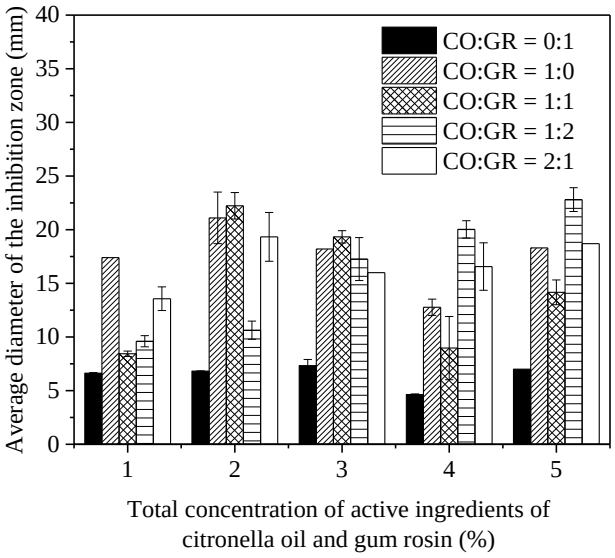


Figure 5. Effect of concentrations and ratios of citronella oil and gum rosin in floor cleaners on the inhibition zone against S. *Thypi.*
CO=citronella oil; GR=gum rosin

The mechanism of action of these compounds occurs by increasing the membrane fluidity and permeability of the microorganisms, resulting in cellular disruption or lysis [5]. Gum rosin is a diterpenoid mainly composed of diphenic acid mainly abietic acid, isopimaric acid, laevoabietat acid, and pimaric acid [10]. Studies showed that diterpenoid compounds, particularly abietic acid have antiviral, antibiotic, and antifungal properties [21]. It is also effective against bacteria, particularly gram-positive bacteria [22]. This might explain why the antimicrobial activity of gum rosin against the strain was relatively low in this study since *S. thypi* is a gram-negative bacteria. The antimicrobial mechanism of diterpenoids, particularly abietic acid, might be attributed to the carboxylic group, which interacts with the lipid component of the bacterial cellular membrane, disrupting the lipophilic cell membrane. As a result, the cell membranes undergo lysis [14,21].
To assess the effectiveness of floor cleaners formulated in this study, the antimicrobial activity of the commercial floor cleaner containing benzalkonium chloride was then investigated. As presented in Figure 6, for all concentrations, the commercial floor cleaner exhibited higher microbial activity against S. *thypi* by 1.4–2.2 times compared to floor cleaners containing gum rosin alone. In contrast, its microbial activity was 27–56 % lower compared to floor cleaner containing citronella oil alone. All formulas using a combination of citronella oil and gum rosin showed higher microbial activity by 1.1–2.4 times than formulas using benzalkonium chloride. The highest microbial activity was achieved at the formula of 5% of citronella oil and gum rosin with a ratio of 1:2. Under these conditions, the average diameter of the inhibition zone was 22.8±1.1 mm, not significantly different compared to the formula with the concentration of citronella oil and gum rosin of 2% with a ratio of 1:1. According to the work of Söderberg et al. (1990), they found that abietic acid could be chemically converted in agar plates seeded with *Escherichia coli* and *Staphylococcus aureus*. Abietic acid was oxidized during the experiment to the most stable abietic type of acid, namely dehydroabietic acid, which had a slight antibacterial effect against some strains of *Bacillus subtilis* but no effect towards *S. aureus*, gram-negative bacteria, or fungi. This finding might explain why in this study, the increasing gum rosin ratio did not increase the inhibition zone significantly against *S. thypi* [23].
The inhibition zone for a floor cleaner containing 2% citronella oil and gum rosin at a ratio of 1:1 was 22.2 ± 1.2 mm. Meanwhile, when using 5% citronella oil and gum rosin at a ratio of 1:2, the zone of inhibition was 22.8 ± 1.1 mm. It shows that increasing the concentration of antimicrobial agents by 2.5 times results in an increase in the inhibition zone of only 2%. Therefore, a concentration of antimicrobial agents of 2% with a ratio of 1:1 is considered the optimum condition.

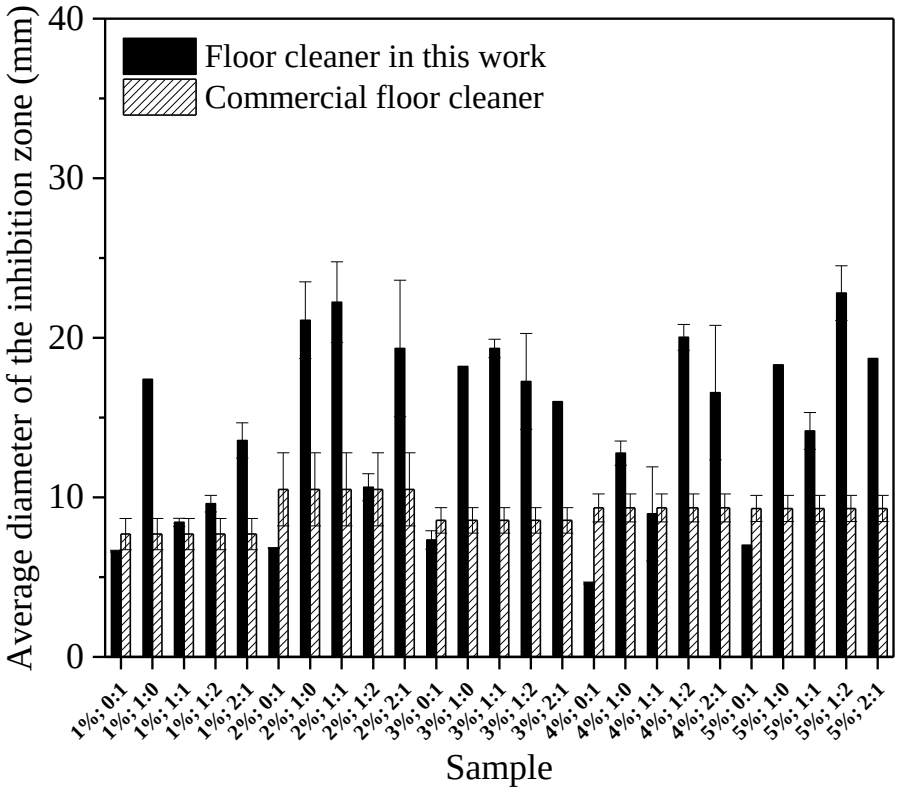


Figure 6. Comparison of the inhibition zone diameter of the floor cleaners containing citronella oil and gum rosin with the commercial floor cleaner containing benzalkonium chloride.

4. CONCLUSION

In this work, the production of floor cleaner liquid using citronella oil and gum rosin as antimicrobial agents has been demonstrated. Floor cleaners have a relatively stable pH and emulsion in hard water. Floor cleaners containing citronella oil and gum rosin developed in this work (concentration 2%, ratio 1:1) had antimicrobial activity against *Salmonella thypi* 2.1 times higher than the commercial floor cleaner containing benzalkonium chloride (concentration 2%). Citronella oil and gum rosin have great potential as antimicrobial agents for floor cleaning products.

ACKNOWLEDGMENT

Authors would like to thank the Faculty of Engineering, University of Brawijaya for financially supporting this research.

REFERENCES

[1] Thermo Fisher Scientific, Safety Data Sheet of benzalkonium chloride, pp. 1–8, 2021, https://www.fishersci.com.

[2] Sasol Chemicals (USA) LLC, Product Stewardship Summary Cresylic Acid, pp.1–7, https://www.sasol.com.

[3] Sasol Chemicals (USA) LLC, Safety Data Sheet Ethoxylated Alcohol, pp. s1-11, 2020, https://sasoltechdata.com.

[4] Sigma-Aldrich, Safety Data Sheet Pine needle oil, pp. 1–9, 2021, https://www.sigmaaldrich.com.

[5] B. G. Cunhaa, C. Duqueb, K. S. Caiaffab, L. Massunarib, I. A. Catanozea, D. M. dos Santosa, S. H. P.

de Oliveirac, A. M. Guiotti, Cytotoxicity and antimicrobial effects of citronella oil (Cymbopogon nardus) and commercial mouthwashes on *S. aureus* and *C. albicans* biofilms in prosthetic materials, *Arch. Oral Biol.*, vol. 109, pp. 414–420, 2020.

[6] J. Kim, M. R. Marshall, C-i Wei, Antibacterial activity of some essential oil components against five foodborne pathogens, *J. Agric. Food Chem*, vol. 43, no. 11, pp. 2839–2845, 1995.

[7] M. Oussalah, S. Caillet, L. Saucier, M. Lacroix, Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella Typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*, *Food Control*, vol. 18, no. 5, pp. 414–420, 2007.

[8] F. N. Victoria, C. S. Radatz, M. Sachini, R. G. Jacob, D. Alves, L. Savegnago, G. Perin, A. S. Motta, W. P. Silva, E. J. Lenardão, Further analysis of the antimicrobial activity of α-phenylseleno citronellal and α-phenylseleno citronellol, *Food Control*, vol. 23, no. 1, pp. 95–99, 2012.

[9] B. Teixeira, A. Marquesa, C. Ramosa, N. R. Neng, J. M. F. Nogueira, J. A. Saraiva, M. L. Nunes, Chemical composition and antibacterial and antioxidant properties of commercial essential oils, *Ind. Crops Prod.*, vol. 43, no. 1, pp. 587–595, 2013.

[10] M. Natsir, M. Nurdin, A. Ansharullah, M. Z. Muzakkar, E. Trimutia, I. Irwan, L. O. A. Salim, S. Salmah, M. Maulidiyah, The technique for separation and purification of gondorukem (gum rosin) from pine gum (pinus merkusii) with a simple distillation method, *J. Phys. Conf. Ser.*, vol. 1899, no. 012038, 2021.

[11] J. J. W. Coppen, G. A. Hone, Gum Naval Stores: Turpentine and Rosin from Pine Resin, Food and Agriculture Organization of the United Nations, 1995.

[12] K. C. d. S. Rodrigues-Correa J. C. de Lima, A. G. Fett-Neto, Pine oleoresin: tapping green chemicals, biofuels, food protection, and carbon sequestration from multipurpose trees, *Food energy Secur.*, vol. 1, no. 2, pp. 81–93, 2012.

[13] A. Sukarno, A. Zairina, Y. Quarta, R. Kurniasari, S. Sumardi, A. S. Leksono, Yield and components of pine (*Pinus merkusii*) turpentine among age class differences tapping by borehole method, *Indones. J. Environ. Sustain. Dev.*, vol. 11, no. 1, pp. 44–48, 2020.

[14] A. Urzúa, M. C. Rezende, C. Mascayano, L. Vásquez, A structure-activity study of antibacterial diterpenoids, *Molecules*, vol. 13, no. 4, pp. 882–891, 2008.

[15] A. Sipponen, Coniferous resin salve, ancient and effective treatment for chronic wounds: laboratory and clinical studies, Ph.D. Dissertation, Dept. of Orthopedics and Traumatology, Helsinki Univ., Helsinki, Finland, 2013.

[16] I. Husna, S. Khaira, Pembuatan cairan pembersih lantai dari bahan alami buah lerak (*Sapindus rarak DC*) aroma sereh wangi, *Proceeding of 4th International Conference on Education*, pp. 149–152, 2019.

[17] L. Humaira, S. Srikandi, Pemanfaatan potensi limbah kulit manggis dalam pembuatan pembersih lantai berbasis *zero waste*, *J. IKRA-ITH Ekon.*, vol. 4, no. 2, pp. 69–72, 2021.

[18] E. R. Desfitri, R. Desmiarti, S. Y. Verdana, A. Amanda, Pembuatan cairan pembersih lantai dengan memanfaatkan minyak atsiri dan hidrosolnya, *React. J. Res. Chem. Eng.*, vol. 3, no. 1, pp. 28–35, 2022.

[19] Clinical and Laboratory Standards Institute, Performance standards for antimicrobial disk susceptibility tests: Approved standard – 11th ed., CLSI document M02-A11, vol. 32, no. 1. 2012.

[20] Badan Standarisasi Nasional, Standar Nasional Indonesia Pembersih lantai, SNI 06-1842-1995.

[21] E. Buommino, A. Vollaro, F. P. Nocera, F. Lembo, M. DellaGreca, L. De Martino, M. R. Catania, Synergistic effect of abietic acid with oxacillin against methicillin-resistant *Staphylococcus pseudintermedius*, *Antibiotics*, vol. 10, no. 80, pp. 1–12, 2021.

[22] M. Shuaib, A. Ali, M. Ali, B. P. Panda, M. I. Ahmad, Antibacterial activity of resin rich plant extracts, *J. Pharm. Bioallied Sci.*, vol. 5, no. 4, pp. 265–269, 2013.

[23] T. A. Söderberg, R. Gref, S. Holm, T. Elmros, G. Hallmans, Antibacterial activity of rosin and resin acids in vitro, *Scand. J. Plast. Reconstr. Surg. Hand Surg.*, vol. 24, no. 3, pp. 199–205, 1990.