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The Antibacterial Activity of Infusion of Averrhoa bilimbi L Fruits and Cananga odorata Flowers against Frequently Pathogenic Bacteria

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Abstract

Infectious diseases due to opportunistic bacteria and pathogens are still a health problem. Transmission can be prevented by using an alcohol-based antiseptic liquid or naturally. *Averrhoa bilimbi* L. fruit plants and *Cananga odorata* flowers contain bioactive compounds that can inhibit bacterial growth. This study aimed to analyze the infusion activity of *Averrhoa bilimbi* fruit and *Cananga odorata* flower in single and combined dosage forms against several frequently pathogenic bacteria in vitro. This posttest-only experimental study with a control group design used the paper disc diffusion method. Observation parameters were the diameter of the inhibition zone on the test bacteria after infusion treatment of 50%, 75%, and 100% *A.bilimbi* fruit and 100% *C.odora* flowers, as well as 70% alcohol. The results showed that the diameter of the inhibition zone in the combination treatment of *A.bilimbi* and *C.odorata* infusion was significantly different from that of the single preparation ($p<0.05$). The combination of 100% *A.bilimbi* and 100% *C.odorata* (ratio 1:1) produced the greatest effect and was equivalent to 70% alcohol. The average inhibition of the combination infusion of *Staphylococcus aureus* (17.36mm) and *Staphylococcus epidermidis* (17.57mm) was greater than that of *Escherichia coli* (14.48 mm) and *Salmonella enterica serovar* Typhi (14.12mm). In conclusion, the infusion of *Averrhoa bilimbi* fruit and the *Cananga* odorata flower had antibacterial activity, and the combined preparation produced a better inhibitory effect than the single preparation.

Keywords: antibacterial activity, *Averrhoa bilimbi L.*, *Cananga odorata*, infusion, single preparation, combination preparation

Introduction

Infectious diseases related to one's hygiene are still a health problem in the community, especially for those who live on riverbanks (Budiarti et al., 2022). The most common causative agents found were opportunistic and obligate pathogens. Generally, infection transmission often occurs due to the use of water that has been contaminated with bacteria and direct contact with hands or the presence of bacterial contaminants on the skin surface (Budiarti, Isnaini, et al., 2021; Wulansari & Parut, 2019). The results of the study which identified the types of bacteria in hand swabs and feces samples from people living around the riverbanks of Banjarmasin City, found the types of bacteria *Staphylococcus aureus (S.aureus), Staphylococcus epidermidis (S.epidermidis), Escherichia coli (E.coli)* in hand swab samples and *Salmonella enterica serovar* Typhi in stool samples (Kurniati et al., 2019; Yulia Budiarti et al., 2017.).

Prevention of transmission of bacteria from person to person, namely by the use of antiseptics; aimed at reducing or killing bacterial colonization found on the surface or skin tissue (Budiarti, Isnaini, et al., 2021). The most commonly used antiseptics are alcohol-based. Alcohol is a bactericide that works to damage cell membranes, inhibits enzyme performance, and denatures bacterial cell proteins (Asngad et al., 2018). The disadvantage is that the use of alcohol in the long term causes the skin to feel burning, irritated, and dry skin (Fardan & Harimurti, 2018). An effort to reduce the impact of alcohol use

is to use natural antiseptic preparations. Antiseptic product innovations can be developed from herbs containing antibacterial compounds, intended to reduce the use of chemical-based drugs and their negative effects (Budiarti *et al*., 2022).

Biodiversity in the form of herbal plants in the territory of Indonesia has been widely used as phytopharmaca ingredients, including those sourced from the fruit of *Averrhoa bilimbi* L (*A. bilimbi*) known as star fruit wuluh and *Cananga odorata (C. odorata)* or ylang flower. In Indonesia Averrhoa bilimbi (Family Oxalidaceae) is a plant that is commonly used as traditional medicine and cooking ingredients. (Dewi et al., 2019). In the people of South Kalimantan, the juice of *A.bilimbi* fruit is also used to reduce the fishy smell on hands when processing fish-based food. *C.odorata* flowers are generally very commonly used in funeral and religious ceremonies, namely the dosage form of the flower water gives a fragrant and fresh aroma.

A. bilimbi plants are used for the treatment of various diseases; Infusion and decoction of the leaves are used as antibacterial, antiscorbutic, astringent, fever medicine, rectal inflammation, and diabetes. The fruit part is used more often; grated fruit is used as an acne remedy; while fruit juice is used in treating scurvy, biliary colic, whooping cough, hypertension, obesity, and diabetes (Alhassan & Ahmed, 2016; Dewi et al., 2019). *A.bilimbi* fruit contains flavonoid compounds (apigenin and luteolin)(Zarwinda et al., 2022) ; and tannins, coumarins, and terpenes (Alhasan and Qamar, 2017). The content in *C.odorata* flowers is flavonoid compounds, tannins, saponins, and steroids (Putri et al., 2020). Water extract and chloroform extract from the leaves and fruit of *A. bilimbi* produced an effect on *S.aureus, S.epidermis, B.cereus, Salmonella enterica serovar* Typhi*, C.freundii, A.hydrophila, P.vulgaris*, and *K.rhizophila* (Alhassan & Ahmed, 2016). In various solvents, *A.bilimbi* fruit extract has an effect on *S. aureus* and *Salmonella enterica serovar* Typhi (Maryam et al., 2015); *E.coli, P.aeruginosa, S.typhimurium, S.aureus,* and *V.parahaemolytius* (Seebaluck-Sandoram et al., 2019); also against gram-negative and gram-positive multi-drug resistance (MDR) bacteria (Prastiyanto et al., 2020). *A. bilimbi* extract was able to reduce the number of bacterial colonies on the hands (Santoso et al., 2020).

C.odorata flowers can be used as a basic ingredient for making natural cosmetics and medicine; they have been used as aromatherapy in the manufacture of perfumes and liquid soap (Anggia et al., 2018). The efficacy of *C.odorata* flower as a medicine, namely skin disease medicine, mosquito repellent, asthma medicine, antioxidant, and antibacterial (Dusturia, N., S.R. Hikamah, 2016). *C.odorata* flowers can be used in the manufacture of transparent soap (Maulidya et al., 2020). The addition of essential oils in the content of hand soap can increase the antimicrobial effect (Heriyani et al., 2021). The ethanolic extract of *C.odorata* flowers has an effect on *S.aureus, S.epidermidis, B.cereus* and *E.coli* (Yulinah *et al*., 2014). Its essential oil has a good effect on *B.subtilis, S.aureus, S.enteritidis*, and *E.coli* (Anggia et al., 2018). Hand sanitizer gel *C.odorata* 0.5%, 1.25%, 2.5%, and 5% were able to inhibit *E.coli* and *S.aureus* (Herlina et al., 2020).

Antibacterial preparations can be used singly and in combination; A good combination of extracts has a synergistic effect. The combination of infusion at the appropriate concentration can produce better effectiveness against bacteria and fungi (Budiarti, Isnaini, et al., 2021). The combination of *M.calabura* leaf extract and *A.bilimbi* 10%-20%, the ratio of 1:2, 2:1, 1:3, and 3:1 resulted in moderate inhibition (Pramiastuti et al., 2020). (Tadtong et al., 2012) stated that a mixture of ylang flower oil extract, lavender, and clary sage (ratio 3:4:3) produced good effects on *S.aureus, S.epidermidis, E.coli, P.aeruginosa,* and *C.albicans* (Tan et al., 2015).

The presence of antibacterial content in *A.bilimbi* and *C.odorata* flowers allows it to be developed as an antiseptic preparation, the effectiveness of which is known through the phenol coefficient test(Budiarti, Wydiamala, et al., 2021). The phenol coefficient of the combination of *A.bilimbi* fruit infusion and *C.odorata* flowers on *S.aureus* was different from that of *Salmonella enterica serovar* Typhi (Budiarti, Isnaini, et al., 2021). Continuation of the research is reported in this study; this study aimed to analyze the antibacterial activity of the infusion of *Averrhoa bilimbi* L. fruit and *Cananga odorata* flowers, in single and combination preparations against several frequently pathogenic bacteria.

Methods

This experimental study used a posttest only with a control group design, this was approved by the ethical commission of the Faculty of Medicine, University of Lambung Mangkurat; letter number: 753/KEPK-FKULM/EC/VIII/2021. The research was conducted at the Pharmacology and Microbiology Laboratory, Faculty of Medicine, University of Lambung Mangkurat Banjarbaru, from October to November 2021.

Materials

The test plants were from Banjrbaru, South Kalimantan, and were identified as *Averrhoa bilimbi* L. and *Cananga odorata*. The selected *A.bilimbi* fruit material is fresh green, not too ripe, and soft; while the yellow petals of *C.odorata* are selected fresh and picked in the morning. The test bacteria isolates were collected from the Microbiology Laboratory of the Faculty of Medicine, ULM, namely *Staphylococcus aureus* ATCC 29523, *Staphylococcus epidermidis* ATCC 35983, *Escherichia coli* ATCC 25922, and *Salmonella enterica serovar* Typhi ATCC 19430.

Laboratory Analysis

Infusion of Averrhoa bilimbi and Cananga odorata

The research materials that have been obtained, namely *A.bilimbi* fruit and *C.odorata* flowers were washed with running water until clean to remove all dirt attached to these materials. Then the fruit and flowers were dried in the sun using a black cloth cover. This was done so that the test plant material was protected from secondary metabolite degradation due to sunlight. After that, the dry ingredients were mashed with a blender to reduce the size.

In each test plant, an infusion was made. The trick was to add sterile distilled water to the test material until a 100% level was

obtained, placed it in a beaker glass container and placed it on a hot water bath/infusion pot (90°C) and he for 15 minutes, stirring occasionally. The results of the infusion preparation were filtered through filter paper while hot, then sterile water solvent was added to obtain 100% concentration. Then, several preparations of fruit infusion of *A.bilimbi* 50%, 75%, and 100%, as well as flower infusion of *C.odorata* 100% were made. Combination preparations or mixtures were made by mixing every 2 infusion treatments in a ratio of 1:1 .

Antibacterial Activity Test (Disk Paper Diffusion Method)

Prepared stock of test bacteria; 3-5 bacterial colonies were taken and put aseptically into 5 mL of Brain Heart Infusion (BHI) liquid media. The bacterial suspension was incubated for 2-6 hours at 37°C. Then, sterile saline was added until the turbidity was equal to the standard Mc.Farland 1,5 x 10^8 CFU/mL.

Several 6mm paper discs were prepared to be filled with treatment. Infusion at various test concentrations, 70% alcohol, and sterile distilled water were placed in a test tube. In each test tube, several paper discs were dipped, then placed on the surface of the Mueller Hinton Agar (MHA) media with the help of sterile tweezers with a slight emphasis so that the paper discs adhered well, then the media was incubated 37°C for 48 hours. The diameter of the inhibition zone or the clear zone formed around the paper disc was measured using a caliper.

Statistic Analysis

Research data from three repetitions had a normal and homogeneous distribution. Data were analyzed using the One-way Anova test and Duncan test at a 95% confidence level.

Results

Infusion of A. bilimbi fruit and *C.odorata* flower in single and combined dosage forms resulted in an inhibitory effect on several test bacteria. Figure 1 showed the diameter of the inhibitor produced by the infusion of *A.bilimbi* and *C.odorata* was directly proportional to the concentration. The inhibition zone for the treatment of a single infusion preparation was under the combination treatment of infusion and 70% alcohol treatment.

(Davis & Stout, 1971), stated that the antibacterial activity can be classified as a) very strong, with a diameter of the inhibition zone is 20 mm or more; b) strong, zone of inhibition 10-20 mm; c) medium inhibition zone, 5-10 mm; and d) weak, the zone of inhibition is 5 mm or less. In this study, the antibacterial power analysis based on statistical analysis is shown in Table 1.
The smallest average inhibition zone was from the infusion of 50% *A.blimbi* against Salmonella enterica serovar Typhi which was 7.76mm. The widest zone of inhibition was the combination of 100% *A.bilimbi* and 100% *C.odorata* infusion against *S.epidermidis,* which was 17.57mm. The antibacterial power of a single infusion is in the moderate to strong category; while the combination treatment of infusion and 70% alcohol was strong. In general, the infusion of *A. bilimbi* fruit and *C.odorata* flowers produced an inhibitory effect on Gram-positive bacteria *S.aureus* and *S.epidermidis* greater than that of Gram-negative bacteria (*E.coli* and *Salmonella enterica serovar* Typhi).

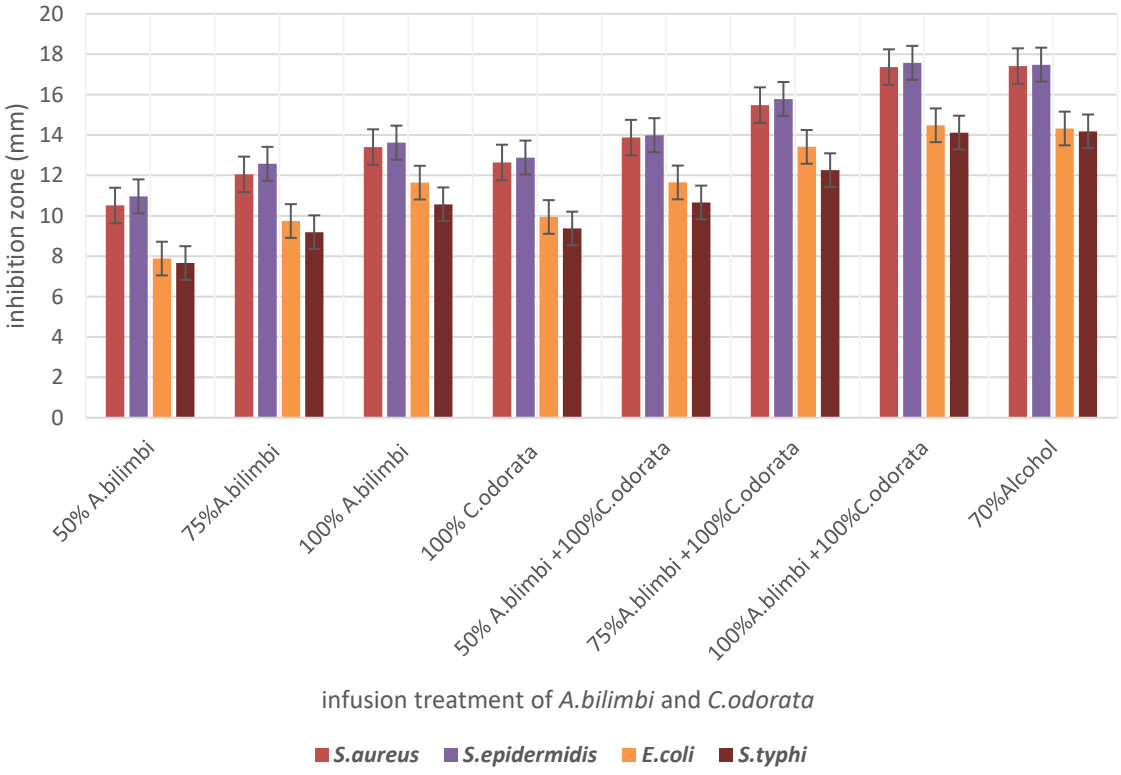

Figure 1. Inhibitory diameter of infusion treatment of *Averrhoa bilimbi* fruit and *Cananga odorata* flower against test bacteria

Table 1. The average diameter of the infusion inhibition zone of *A.bilimbi* and *C.odorata* fruit against several test bacteria

Treatment	inhibition zone diameter (mm)			
	S.aureus	*S.epidermidis*	*E.coli*	*Salmonella enterica serovar* Typhi
Infusion of100% *C.odorata**	12,64±0,18[a]	12,88±0,06[a]	9,94±0,03[a]	9,73±0,10[a]
Infusion of 50% *A.bilimbi**	10,51±0,16[b]	10,96±0,07[b]	7,88±0,03[b]	7,76±0,15[b]
Infusion of 75% *A.bilimbi**	12,05±0,10[c]	12,57±0,35[c]	9,47±0,09[c]	9,19±0,07[c]
Infusion of 100% *A.bilimbi**	13.40±0,15[d]	13,62±0,10[d]	11,64±0,06[d]	10,57±0,09[d]
Combination of 50%A.bilimbi*+100%*C.odorata*	13,87±0,05[e]	13,99±0,15[e]	11,65±0,14[e]	10,66±0,06[e]
Combination of 75%A.bilimbi*+100%*C.odorata*	15,48±0,03[f]	15,78±0,09[f]	13,41±0,14[f]	12,2,07[f]
Combination of 100%A.bilimbi*+100%*C.odorata	17,36±0,05[g]	17,57±0,35[g]	14,48±0,03[g]	14,12±0,04[g]
70%Alcohol 70% (control)	17,41±0,03[g]	17,48±0,03[g]	14,32±0,03[g]	14,18±0,04[g]

* $p < 0,05$, significant differences between the treatment infusion and the control

Based on the results of the Anova test and Duncan's test (Table 1), there was a significant difference ($p<0.05$) between the combination treatment of 100% *A.bilimbi* and 100% *C.odorata* infusions and 70% alcohol treatment with all single-dose treatments and with a combination of *A.bilimbi* below 100%. The combined effect of 100% infusion is equivalent to that of 70% alcohol. In general, the combined effect of the infusion is better than the single dosage form.

Discussion

The results of this study showed that the infusion of *A.bilimbi* fruit and *C.odorata* flowers had antibacterial active substances against all the bacteria tested, this is like several previous research reports. The administration of the liquid extract of *A.bilimbi* fruit affected both Gram-positive and Gram-negative bacteria; such as in S*.aureus, B.cereus, E.coli, P.aeruginosa,* and *Salmonella spp*. (Mokhtar & Abd Aziz, 2016); on S*.epidermis, B.cereus, K.rhizophila, C. diphtheria, Salmonella enterica serovar* Typhi*, C.fuendii, A.hydrophila*, and *P.vulgaris* (Sugiharto, 2020). Ripe fruit extract of *A.bilimbi* produces a better effect than young fruit (Mokhtar & Abd Aziz, 2016). *C.odorata* flowers produce antibacterial compounds (Putri et al., 2020). The extract has an effect on *S.aureus, S.epidermidis, B.cereus* and *E.coli* (Yulinah *et al*., 2014); and on *S. aureus* and *E.coli* (Herlina et al., 2020).

Increasing the concentration of the combination treatment of *A.bilimbi* fruit and *C.odorata* flower infusion can increase the dissolved secondary compounds; thereby increasing its activity as an antibacterial. Phytochemical compounds can act as a shield against infectious diseases (Shahat & Marzouk, 2013). Important compounds such as tannins, flavonoids, alkaloids, tannins, and terpenoids have been shown to exhibit quite strong antibacterial activity (Broniatowski et al., 2015; Hasanuzzaman et al., 2013).

In the study, better antibacterial activity was obtained from the combination of a 100% infusion of *A.bilimbi* fruit and 100% *C.odorata* flower. Compounds in combination preparations with appropriate ratios, can synergize and produce better effects; such as compounds in *A.bilimbi* are flavonoids, saponins, triterpenoids, and oxalic acid (Rachmawati et al., 2017; Sugiharto, 2020); while in *C.odorata* are essential oils, flavonoids, and saponins (Anggia et al., 2018; Dusturia, N., S.R. Hikamah, 2016).

Several previous studies also reported a greater inhibitory effect than combination preparations. Combination of *A.bilimbi* and *A.vera* extract gel on *S.aureus* (Zarwinda et al., 2022). The linalool and linalyl acetate compounds in cananga flowers with lavender and clary sage are components that play a role in the combination of essential oils (Tadtong et al., 2012). The combination of herbs can increase the synergistic effect, thus providing a better inhibitory effect (Tan *et al*., 2015). The synergistic effect of the combination of extracts enriched with different constituents also enhances the biological activity; for example, the tannin-enriched EtOAc fraction from the roots of the *Cochlospermum regium* plant produced the best combination as an antimicrobial (Carvalho et al., 2018).

In this study, an aqueous solvent was used as an infusion preparation. Water can dissolve some polar compounds contained in *A.bilimbi* fruit and *C.odorata* flowers to work to inhibit the test bacteria. Water is a universal solvent that can dissolve secondary compounds of flavonoids, phenols, anthraquinones, alkaloids, and tannins (Fibonacci & Hulyadi, 2018). Secondary compounds can destroy bacterial cell walls and inhibit bacterial growth (Abuga et al., 2020; Prastiyanto et al., 2020). The compounds contained in *A.bilimbi* are flavonoids (Khalid et al., 2019; Prastiyanto et al., 2020), tannins, saponins (Arisanty & Dewi, 2018; Maryam et al., 2015), alkaloids and terpenoids (Prastiyanto et al., 2020). Secondary compounds found in *C.odorata* flowers are flavonoids, tannins, saponins, steroids (Herlina *et al*., 2018), phenolic compounds (Rahardhian et al., 2019), and essential oils (Putri et al., 2020).

Compounds that are often important in overcoming pathogenic microbes are flavonoids. Flavonoids inhibit the diffusion of free radicals and protect many oxidative factors by interacting directly with pathogenic cellular membranes. Flavonoids are located in the lipid bilayer and regulate non-enzymatic and enzymatic lipid peroxidation mechanisms. Flavonoids also inhibit the activity of the enzymes involved; Free radical generation includes lipoxygenase, cyclooxygenase, microsomal monooxygenase, xanthine oxidase, and glutathione S-transferase (GST) (Khalid et al., 2019). These compounds damage the permeability of the walls, microsomes, and lysosomes of bacterial cells by exploiting the polarity difference between lipids as constituents of bacterial cells and the alcohol groups of flavonoid compounds. (Arisanty & Dewi, 2018), The lipophilic nature of flavonoid compounds will inhibit cell membrane function, nucleic acid synthesis, and bacterial energy

metabolism (Achwandi & Khoiriyati, 2015). Differences in polarity can block the attachment and formation of biofilms, inhibit porins in cell membranes, and change cell membrane permeability, thereby weakening the pathogenicity of pathogenic bacteria (Sugiharto, 2020).

Alkaloid compounds showed good antibacterial activity on Gram-negative bacteria, Gram-positive bacteria, and fungi. Alkaloids have been gradually applied in clinical practice, and the effect is relatively significant. The types of compounds that include alkaloids are quinoline alkaloids, piperidine alkaloids, polyamine alkaloids, imidazole alkaloids, and peptide alkaloids. The mechanism of action of alkaloids is to disrupt bacterial cell membranes by lipophilic compounds, affect DNA function, inhibit protein synthesis, modify bacterial cell walls, inhibit bacterial metabolism, and inhibit efflux pumps (Wahdaningsih et al., 2014).

Saponin compounds play a role in damaging bacterial cell walls (Sugiharto, 2020). This compound causes leakage of proteins and enzymes in bacterial cells , and inhibits metabolic processes to kill bacteria (Sugiharto, 2020). Tannin compounds can enter bacterial cells through cell walls that have been damaged by the action of other compounds. Furthermore, it will inactivate enzymes and interfere with protein transport in the inner layer of bacterial cells (Akib et al., 2019). Tannins inhibit the reverse transcriptase and DNA topoisomerase enzymes so that bacterial cells are not formed (Qolbi & Yuliani, 2018). Triterpenoid compounds can help destroy cell membranes resulting in bacterial death. According to Wu *et al*., (2013), triterpenoids can react with porins on the outside of the wall which leads to the lysis of the bacterial cell wall (Sugiharto, 2020). Triterpenoids damage the lipid fraction of the cytoplasmic membrane so will interfere with the process of forming a membrane or cell wall

The combination of infusion of *A.bilimbi* fruit and *C.odorata* flower in this study resulted in a wider inhibitory effect on Gram-positive bacteria than against Gram-negative bacteria. The antimicrobial activity of extracts of *A.bilimbi* (Prastiyanto et al., 2020; Sugiharto, 2020) and also extracts of *C.odorata* (Tan et al., 2015) were said to have a better effect on Gram-positive bacteria. In the test results comparing the antibacterial activity of two forms of nanoparticles using *A.bilimbi* extract, it was found that the inhibition zone of *S.aureus* was larger than that of *E.coli* (Lisnawati et al., 2019). The same thing was found in a study that combined 1.5% Cananga odorata essential oil in a transparent solid soap based on VCO and palm oil, which resulted in a better effect on *S.aureus* than *E.coli* (Maulidya et al., 2020). The bacteriostatic activity of each essential oil and perfume is generally better able to inhibit the growth of Gram-positive bacteria (Tan et al., 2015).

Gram-positive bacteria have a simpler cell wall composition so that nothing prevents the entry of antibacterial substances and antimicrobial compound molecules. The cell membrane of gram-negative bacteria contains a high composition of lipopolysaccharide (LPS) and generally produces mucus as a form of self-defense from harmful chemical compounds. Hydrophilic molecules will more easily pass through LPS than hydrophobic ones (Nasution et al., 2019; Tan et al., 2015).

The results of our previous study showed differences in the value of the phenol coefficient of the combination of infusions of *A.bilimbi* fruit and *C.odorata* flowers; against *S.aurues* was found to be higher than against *Salmonella enterica serovar* Typhi (Budiarti *et al*., 2021). In this study, it was proven that the combination treatment of 100% *A.bilimbi* fruit infusion and 100% *C.odorata* flower resulted in a synergistic effect so that the antibacterial power was equivalent to 70% alcohol. Based on these two studies, it is possible that the combined extract of *A.bilimbi* and *C.odorata* can be developed as an antiseptic preparation. Furthermore, the effectiveness of the combined preparation on bacterial biofilm formation and forganoleptic tests can be analyzed.

Conclusion

The infusion of *Averrhoa bilimbi* L. fruit and *Cananga odorata* flower in single and combination preparations had a significant effect in inhibiting the test bacteria. The infusion *of Averrhoa bilimbi* L fruit and *Cananga odorata* flower in the combined dosage form had better antibacterial activity than the single dosage form

Conflict of interest

There is no conflict of interest in this study.

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Author contribution

Lia Yulia Budiarti contributed to the concept of research, data collection, data analysis, and writing of published manuscripts. Erida Wydiamala and Najiya Ulfa contributed to data collection, analysis, and writing the manuscript for publication.

Reference

Abuga, I., Sulaiman, S. F., Abdul Wahab, R., Ooi, K. L., & Abdull Rasad, M. S. B. (2020). In vitro antibacterial effect of the leaf extract of Murraya koenigii on cell membrane destruction against pathogenic bacteria and phenolic compounds identification. *European Journal of Integrative Medicine*, *33*. https://doi.org/10.1016/j.eujim.2019.101010

Achwandi, M., & Khoiriyati, A. (2015). Efektivitas Ekstrak Daun Sirih Merah (Piper Crocatum) Terhadap Kadar Hambat Minimum Dan Kadar Bunuh Minimum Bakteri Salmonella typhi. *Indonesian Journal Pf Nursing Practices*, *2*(1), 1–8.

Akib, N. I., Wulandari, I. W., Suryani, S., & Hanari, H. (2019). FORMULASI GEL HAND SANITIZER ANTIBAKTERI KOMBINASI EKSTRAK RUMPUT LAUT Eucheuma spinosum DAN Eucheuma cotonii ASAL KEPULAUAN WAKATOBI SULAWESI TENGGARA. *Jurnal Fish Protech*, *2*(2), 180. https://doi.org/10.33772/jfp.v2i2.9232

Alhassan, A., & Ahmed, Q. (2016). Averrhoa bilimbi Linn.: A review of its ethnomedicinal uses, phytochemistry, and pharmacology. In *Journal of Pharmacy and Bioallied Sciences* (Vol. 8, Issue 4, pp. 265–271). Wolters Kluwer -- Medknow Publications. https://doi.org/10.4103/0975-

7406.199342

Anggia, M., Mutiar, S., & Arziah, D. (2018). Teknologi Ekstraksi Bunga Kenanga (Cananga Odorata L.) dan Sereh Wangi (Cymbopogon Nardus L.) Sebagai Aroma Terapi Sabun Cair. *Jurnal Daur Lingkungan*, *1*(1), 5. https://doi.org/10.33087/daurling.v1i1.2

Arisanty, A., & Dewi, R. P. (2018). UJI EFEKTIVITAS EKSTRAK AIR BUAH BELIMBING WULUH (Averrhoa bilimbi) TERHADAP PERTUMBUHAN Propionibacterium acnes. *Media Farmasi*, *14*(2), 66. https://doi.org/10.32382/mf.v14i2.601

Asngad, A., R, A. B., & Nopitasari, N. (2018). Kualitas Gel Pembersih Tangan (Handsanitizer) dari Ekstrak Batang Pisang dengan Penambahan Alkohol, Triklosan dan Gliserin yang Berbeda Dosisnya. *Bioeksperimen: Jurnal Penelitian Biologi*, *4*(2), 61–70. https://doi.org/10.23917/bioeksperimen.v4i2.6888

Broniatowski, M., Mastalerz, P., & Flasiński, M. (2015). Studies of the interactions of ursane-type bioactive terpenes with the model of Escherichia coli inner membrane - Langmuir monolayer approach. *Biochimica et Biophysica Acta - Biomembranes*, *1848*(2), 469–476. https://doi.org/10.1016/j.bbamem.2014.10.024

Budiarti, L. Y., Isnaini, I., Dayana, P., Sari, N., & S., N. A. R. (2021). Antimicrobial Activity of Stenochlaena palustris and Sauropus androgynus in Staphylococcus aureus, Escherichia coli and Candidia albicans. *Bioinformatics and Biomedical Research Journal*, *4*(1), 32–38. https://doi.org/10.11594/bbrj.04.01.05

Budiarti, L. Y., Khariyati, L., & Fakhriyadi, R. (2017). The Relationship Between The Existence of Bacterial Type From Hand and Feces with Water Piping on Elementary School Students on The Riverbanks of Kuin in Banjarmasin. *Proceding International Seminar : Development of Tropical Disease Research Based on Wetland and Indonesian Local*, 336–347.

Budiarti, L. Y., Wydiamala, E., & Ulfa, N. (2021). Phenol Coefficient Test Combination Infusion of Cananga odorata – Averrhoa bilimbi L. Against Staphylococcus aureus and Salmonella typhi in Vitro. *Bioinformatics and Biomedical Research Journal*, *4*(1), 19–26. https://doi.org/https://doi.org/10.11594/bbrj.04.01.03

Budiarti, L. Y., Yasmina, A., Nurikwan, P. W., Prayudi, M. O. S., Firisa, M. R., & Kangsudarmanto, Y. (2022). Antibacterial activity of infused peel of kaffir lime, manurun banana, and pineapple against the number of Staphylococcus aureus and Escherichia coli colonies. *IOP Conference Series: Earth and Environmental Science*, *976*(1), 012034. https://doi.org/10.1088/1755-1315/976/1/012034

Carvalho, R. S., Carollo, C. A., de Magalhães, J. C., Palumbo, J. M. C., Boaretto, A. G., Nunes e Sá, I. C., Ferraz, A. C., Lima, W. G., de Siqueira, J. M., & Ferreira, J. M. S. (2018). Antibacterial and antifungal activities of phenolic compound-enriched ethyl acetate fraction from Cochlospermum regium (mart. Et. Schr.) Pilger roots: Mechanisms of action and synergism with tannin and gallic acid. *South African Journal of Botany*, *114*, 181–187. https://doi.org/10.1016/j.sajb.2017.11.010

Davis, W. W., & Stout, T. R. (1971). Disc Plate Method of Microbiological Antibiotic Assay. *Applied Microbiology*, *22*(4), 666–670. https://doi.org/10.1128/am.22.4.666-670.1971

Dewi, P., Ratih, G. A., Burhannuddin, B., & Sudarmanto, G. (2019). In vitro Inhibitory Activity of Ethanolic Fruit Extract from Averrhoa bilimbi L. against Streptococcus pyogenes Bacteria. *Health Notions*, *3*(1), 13–17. https://doi.org/10.33846/hn.v3i1.276

Dusturia, N., S.R. Hikamah, D. S. (2016). Efektivitas Antibakteri Bunga Kenanga (Cananga odorata) dengan Metode Konvensional terhadap Pertumbuhan Staphylococcus aureus. *Bioshe. J Bioshell*, *5*(01), 324–332.

Fardan, I., & Harimurti, S. (2018). Formulasi Sediaan Gel Minyak Atsiri Daun Cengkeh (Syzygium aromaticum (L.) Merr. & L.M.Perry) Sebagai Antiseptik Tangan dan Uji Daya Hambat Terhadap Bakteri Staphylococcus aureus. *PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia)*, *15*(2), 218. https://doi.org/10.30595/pharmacy.v15i2.3001

Fibonacci, A., & Hulyadi, H. (2018). Uji Aktivitas Antimikroba Daun Sirsak (Annona muricata L.) Terhadap Bacillus subtillis dan Eschericia coli. *Walisongo Journal of Chemistry*, *1*(1), 14. https://doi.org/10.21580/wjc.v2i1.2669

Hardiyanti, R., Budiarti, L. Y., & Heriyani, F. (2019). Perbedaan Jumlah Bakteri Tangan Pada Siswa Sekolah Dasar Di Sekitar Bantaran Sungai Lulut Banjarmasin Berdasarkan Teknik Mencuci Tangan. *Jurnal Homeostatis*, *2*(1), 179–184.

Hasanuzzaman, M., Ali, M. R., Hossain, M., Kuri, S., & Islam, M. S. (2013). Evaluation of total phenolic content, free radical scavenging activity and phytochemical screening of different extracts of Averrhoa bilimbi (fruits). *International Current Pharmaceutical Journal*, *2*(4), 92–96. https://doi.org/10.3329/icpj.v2i4.14058

Heriyani, F., Budiarti, L. Y., Nursantari, W., & Apriliani, A. (2021). Hand Soap Activity Against the Number oOf Bacterial Colonies from the Housewife's Hand Swab Samples in a Temporary Landfill in Kelurahan Gadang Banjarmasin. *Berkala Kedokteran*, *17*(2), 87. https://doi.org/10.20527/jbk.v17i2.11665

Herlina, E., Widiastuti, D., & Triadi, A. (2020). POTENSI MINYAK ATSIRI BUNGA KENANGA (CANANGA ODORATA) SEBAGAI ANTIBAKTERIA DALAM SEDIAAN HAND SANITIZER GEL. *EKOLOGIA*, *20*(2), 88–94. https://doi.org/10.33751/ekologia.v20i2.2171

Khalid, M., Saeed-ur-Rahman, Bilal, M., & HUANG, D. feng. (2019). Role of flavonoids in plant interactions with the environment and against human pathogens — A review. In *Journal of Integrative Agriculture* (Vol. 18, Issue 1, pp. 211–230). Elsevier. https://doi.org/10.1016/S2095-3119(19)62555-4

Lisnawati, N., Kumala, S., & Rahmat, D. (2019). Formulation and evaluation of antibacterial activity of nanoparticles ointment preparation using Blimbi extract. *Pharmaceutical and Biomedical Research*. https://doi.org/10.18502/pbr.v4i3.542

Maryam, S., Juniasti, S., & Kosman, R. (2015). UJI AKTIVITAS ANTIBAKTERI EKSTRAK ETANOL BUAH BELIMBING WULUH (Averrhoa bilimbi L.) ASAL KOTA WATAMPONE. *Jurnal Ilmiah As-Syifaa*, *7*(1), 60–69. https://doi.org/10.33096/jifa.v7i1.21

Maulidya, R., Aisyah, Y., & Yunita, D. (2020). *Quality Characteristics and Antibacterial Activity of Transparent Solid Soap with Addition of Cananga Oil (Cananga odorata)*. 112–118. https://doi.org/10.5220/0009957701120118

Mokhtar, S. I., & Abd Aziz, N. A. (2016). Antimicrobial Properties of Averrhoa bilimbi Extracts at Different Maturity Stages. *Journal of Medical Microbiology & Diagnosis*, *5*(3). https://doi.org/10.4172/2161-0703.1000233

Nasution, R., Azwar, A. I., Helwati, H., & Marianne. (2019). Antibacterial Activities of Perfume: Combination Flower Magnolia alba, Cananga odorata and Mimusops elengi L, Fixed with Pogostemon cablin Oil. *Indonesian Journal of Pharmaceutical and Clinical Research*, *2*(1), 19–23. https://doi.org/10.32734/idjpcr.v2i1.814

Pramiastuti, O., Rejeki, D., & Maghfiroh, I. (2020). Uji Antibakteri Kombinasi Ekstrak Daun Belimbing Wuluh (Averrhoa bilimbi L) Dan Daun Kersen (Muntingia calabura L) Terhadap Staphylococcus aureus. *Parapemikir : Jurnal Ilmiah Farmasi*, *9*(2), 33–41. https://doi.org/10.30591/pjif.v9i2.2026

Prastiyanto, M. E., Wardoyo, F. A., Wilson, W., & Darmawati, S. (2020). Antibacterial Activity of Various Extracts of Averrhoa bilimbi against Multidrug Resistant Bacteria. *Biosaintifika: Journal of Biology & Biology Education*, *12*(2), 163–168. https://doi.org/10.15294/biosaintifika.v12i2.23600

Putri, A. meiysa, Muham, A. oca, Anggraini, S., Maisarmah, S., & Yulis, P. A. R. (2020). ANALISIS KUALITATIF KANDUNGAN BUNGA KENANGA (Cananga odorata) SECARA FITOKIMIA dengan MENGGUNAKAN PELARUT ETANOL. *Journal of Research and Education Chemistry*, *2*(1), 43. https://doi.org/10.25299/jrec.2020.vol2(1).4783

Qolbi, N., & Yuliani, R. (2018). Skrining Aktivitas Antibakteri Ekstrak Etanol 70% Sepuluh Daun Tanaman Terhadap Klebsiella Pneumoniae. *Pharmacon: Jurnal Farmasi Indonesia*, *15*(1), 8–18. https://doi.org/10.23917/PHARMACON.V15I1.6169

Rachmawati, A., Suprihadi, A., & Kusdiyantin, E. (2017). IDENTIFIKASI SENYAWA BIOAKTIF PADA ISOLAT BAKTERI BUAH BELIMBING WULUH (Averrhoa bilimbi L.) SEBAGAI AGENSIA HAYATI Xanthomonas oryzae pv. oryzae. *Jurnal Akademika Biologi*, *6*(3), 1–11. https://doi.org/10.2/JQUERY.MIN.JS

Rahardhian, M. R. R., Murti, B. T., Wigati, D., Suharsanti, R., & Putri, C. N. (2019). Solvent concentration effect on total flavonoid and total phenolic contents of Averrhoa bilimbi leaf extract. *Pharmaciana*, *9*(1), 137. https://doi.org/10.12928/pharmaciana.v9i1.8793

Santoso, A., Suryadarma, I. B., Sumari, S., & Sukarianingsih, D. (2020). Pembuatan Sabun Aroma Teraphi untuk Masyarakat Pedesaan. *Jurnal KARINOV*, *3*(1), 5. https://doi.org/10.17977/um045v3i1p5-9

Seebaluck-Sandoram, R., Lall, N., Fibrich, B., Blom van Staden, A., Saleem, H., & Mahomoodally, M. F. (2019). Antimicrobial, antioxidant and cytotoxic evaluation of two underutilised food plants: Averrhoa bilimbi L. (Oxalidaceae) and Phyllanthus acidus L. Skeels (Phyllanthaceae). *Biocatalysis and Agricultural Biotechnology*, *18*. https://doi.org/10.1016/j.bcab.2019.01.036

Shahat, A. A., & Marzouk, M. S. (2013). Tannins and Related Compounds from Medicinal Plants of Africa. In *Medicinal Plant Research in Africa: Pharmacology and Chemistry* (pp. 479–555). Elsevier Inc. https://doi.org/10.1016/B978-0-12-405927-6.00013-8

Sugiharto, S. (2020). The potentials of two underutilized acidic fruits (Averrhoa bilimbi l. and phyllanthus acidus l.) as phytobiotics for broiler chickens. *Journal of Advanced Veterinary Research*, *10*(3), 179–185.

Tadtong, S., Suppawat, S., Tintawee, A., Saramas, P., Jareonvong, S., & Hongratanaworakit, T. (2012). Antimicrobial activity of blended essential oil preparation. *Natural Product Communications*, *7*(10), 1401–1404. https://doi.org/10.1177/1934578x1200701041

Tan, L. T. H., Lee, L. H., Yin, W. F., Chan, C. K., Abdul Kadir, H., Chan, K. G., & Goh, B. H. (2015). Traditional uses, phytochemistry, and bioactivities of Cananga odorata (ylang-ylang). In *Evidence-based Complementary and Alternative Medicine* (Vol. 2015). Hindawi Publishing Corporation. https://doi.org/10.1155/2015/896314

Wahdaningsih, S., Untari, E. K., & Fauziah, Y. (2014). Antibakteri Fraksi n-Heksana Kulit Hylocereus polyrhizus Terhadap Staphylococcus epidermidis dan Propionibacterium acnes. *Pharmaceutical Sciences and Research*, *1*(3), 180–193. https://doi.org/10.7454/psr.v1i3.3490

Wulansari, N. T., & Parut, A. A. (2019). Pengendalian Jumlah Angka Mikroorganisme Pada Tangan Melalui Proses Hand Hygiene. *Jurnal Media Sains*, *3*(1), 7–13. https://jurnal.undhirabali.ac.id/index.php/jms/article/view/694

Zarwinda, I., Adriani, A., Agustina, M., & Negara, W. (2022). Formulasi Sediaan Gel Hand Sanitizer Dari Ekstrak Etanol Daun Belimbing Wuluh (Averhoa Blimbi L .) Kombinasi Gel Lidah Buaya (Aloe Vera) Pendahuluan Covid 19 atau virus corona pertama kali muncul mulut , hidung dan mata . Upaya memutus mata pemerintah. *Jurnal Kedokteran Dan Kesehatan*, *9*(3). https://doi.org/10.32539/JKK.V9I3.18939