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Antibacterial Activity of *Averrhoa bilimbi* and *Cananga odorata* Infusions Against *Cutibacterium acnes*

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ABSTRACT

Acne is a common skin disorder often associated with infection by *Cutibacterium acnes*. The long-term use of synthetic antibiotics such as clindamycin may cause adverse effects and contribute to bacterial resistance, highlighting the need for alternative natural treatments. This study evaluated the antibacterial activity of infusions of belimbing wuluh leaves (*Averrhoa bilimbi* Linn.) and kenanga flowers (*Cananga odorata* (Lam.) Hook.f. & Thomson), administered individually and in combination, against the growth of *C. acnes*. The study was conducted experimentally using seven treatments: positive control (clindamycin), negative control (sterile distilled water), single infusions of belimbing wuluh leaves and kenanga flowers, and combination infusions at ratios of 2:1, 1:2, and 1:1. Antibacterial activity was assessed using the disc diffusion method. All infusion treatments showed antibacterial activity in the moderate category, with mean inhibition zones of approximately 6-7 mm. Under the present disc diffusion conditions, the combination infusions did not demonstrate greater antibacterial activity than the corresponding single infusions. Inhibition zones of the infusion treatments tended to decrease after 48 hours of incubation, which may reflect reduced persistence of activity, regrowth, compound instability, or other diffusion-related factors. These findings indicate that belimbing wuluh leaf and kenanga flower infusions possess antibacterial activity against *C. acnes* and may serve as potential natural anti-acne agents, although further studies using MIC/MBC, checkerboard, time-kill, and stability testing are required to clarify their interaction and antibacterial characteristics.

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ABSTRAK

Jerawat merupakan gangguan kulit yang umum terjadi dan sering dikaitkan dengan infeksi bakteri *Cutibacterium acnes*. Penggunaan antibiotik sintetis seperti klindamisin dalam jangka panjang dapat menimbulkan efek samping dan berkontribusi terhadap resistensi bakteri, sehingga diperlukan alternatif pengobatan alami. Penelitian ini bertujuan mengevaluasi aktivitas antibakteri infusa daun belimbing wuluh (*Averrhoa bilimbi* Linn.) dan bunga kenanga (*Cananga odorata* (Lam.) Hook.f. & Thomson), baik secara tunggal maupun kombinasi, terhadap pertumbuhan *C. acnes*. Penelitian dilakukan secara eksperimental dengan tujuh perlakuan, yaitu kontrol positif (klindamisin), kontrol negatif (akuades steril), infusa tunggal daun belimbing wuluh dan bunga kenanga, serta kombinasi infusa dengan perbandingan 2:1, 1:2, dan 1:1. Aktivitas antibakteri diuji menggunakan metode difusi cakram. Seluruh perlakuan infusa menunjukkan aktivitas antibakteri dalam kategori sedang, dengan diameter zona hambat rata-rata sekitar 6-7 mm. Pada kondisi uji difusi cakram yang digunakan, kombinasi infusa tidak menunjukkan aktivitas antibakteri yang lebih besar dibandingkan infusa tunggal yang bersesuaian. Zona hambat pada perlakuan infusa cenderung menurun setelah 48 jam inkubasi, yang dapat mencerminkan penurunan persistensi aktivitas, pertumbuhan kembali bakteri, ketidakstabilan senyawa aktif, atau faktor lain yang berkaitan dengan proses difusi. Temuan ini menunjukkan bahwa infusa daun belimbing wuluh dan bunga kenanga memiliki aktivitas antibakteri terhadap *C. acnes* dan berpotensi dikembangkan sebagai bahan alami anti-jerawat, namun penelitian lanjutan menggunakan uji MIC/MBC, checkerboard, time-kill, dan uji stabilitas masih diperlukan untuk memperjelas sifat interaksi dan karakteristik antibakterinya.

Kata Kunci: *Cutibacterium acnes*; *Averrhoa bilimbi*; *Cananga odorata*; Difusi cakram; Infusa herbal; Anti-jerawat

1. Introduction

Acne, commonly known as acne vulgaris, is a common skin disorder that frequently occurs during adolescence and early adulthood. The primary cause of acne is the bacterium *Cutibacterium acnes*, which colonizes the sebaceous glands of the skin. In addition, external factors such as stress, climate, cosmetics, diet, and medications may also contribute to acne development [1], [2]. Acne treatment generally involves the use of topical or oral antibiotics, light therapy, and active compounds such as benzoyl peroxide, which possesses antibacterial properties [3]. Antibiotics are widely used due to their ability to reduce inflammation and control bacterial infection [4]. Among these, clindamycin is one of the most commonly prescribed antibiotics for mild to moderate acne. Clindamycin inhibits the growth of *C. acnes* and has been reported to be as effective as benzoyl peroxide when applied topically [5]. Hamzah et al. reported that clindamycin produced an average inhibition zone of 21.83 mm against *C. acnes* [6]. However, the prolonged use of antibiotics may lead to reduced effectiveness and increase the risk of antibiotic resistance. Therefore, the exploration of alternative treatments derived from natural sources is necessary.

Belimbing wuluh (*Averrhoa bilimbi*) has long been used in traditional medicine to treat various conditions, including acne and skin infections [7]. This plant contains bioactive compounds such as flavonoids, saponins, and tannins, which contribute to its antibacterial activity. These compounds are known to disrupt bacterial cell walls,

interfere with membrane permeability, and inhibit enzymatic activity. Waluyo et al. reported that the ethanol extract of belimbing wuluh leaves exhibited antibacterial activity against *C. acnes* [8]. Similarly, kenanga flowers (*Cananga odorata*) contain flavonoids, tannins, saponins, and steroids, which also exhibit antibacterial properties [10]. These compounds may act through multiple mechanisms, including protein denaturation, membrane disruption, and inhibition of bacterial metabolism. Sinaga et al. reported antibacterial activity of kenanga flower extract against *C. acnes* [11].

The combination of these two plant extracts is hypothesized to produce enhanced antibacterial activity due to complementary and potentially synergistic mechanisms. The presence of overlapping phenolic compounds, such as flavonoids and tannins, may strengthen membrane disruption, while saponins can increase cell permeability, facilitating the entry of other active compounds into bacterial cells. This multi-target mode of action may lead to synergistic or additive antibacterial effects compared to single extracts.

The combination of antibacterial agents may result in synergistic, antagonistic, or additive effects. Previous studies have shown that combining plant extracts can enhance antibacterial activity compared to single extracts. Otieno et al. reported that combinations of plant extracts produced greater antibacterial inhibition [12], while Budiarti et al. found that a combination of belimbing wuluh leaf infusion and kenanga flower infusion was the most effective against several pathogenic bacteria [13].

Despite these findings, previous studies have primarily focused on the antibacterial activity of single plant extracts or their general combinations, without specifically evaluating the interaction and effectiveness of combined infusions of *Averrhoa bilimbi* leaves and *Cananga odorata* flowers against *C. acnes*. Furthermore, the potential synergistic effects of these combinations remain insufficiently explored. Therefore, this study aims to evaluate the antibacterial activity of combined infusions of belimbing wuluh leaves and kenanga flowers against *C. acnes*, with an emphasis on assessing their potential synergistic effects as a natural alternative for acne treatment.

2. Methods

Study Design

This laboratory-based experimental study evaluated the antibacterial activity of single and combined infusions of *Averrhoa bilimbi* leaves and *Cananga odorata* flowers against *Cutibacterium acnes*. The study consisted of seven treatment groups, each tested in triplicate: a positive control, a negative control, two single-infusion groups, and three combination-infusion groups. The positive control was clindamycin solution (300 mg/10 mL), and 20 µL of the solution was applied to each sterile paper disc before testing. Sterile distilled water was used as the negative control. The plant-based treatment groups consisted of a single infusion of *Averrhoa bilimbi* leaves, a single infusion of *Cananga odorata* flowers, and three combinations of the two infusions prepared at ratios of 2:1, 1:2, and 1:1 (v/v). In the present study, the term *100% infusion* refers to the undiluted filtrate obtained after the infusion process, without further dilution or volume adjustment.

Materials

The equipment used in this study included an analytical balance, knife and cutting board, beakers, measuring cylinders, Erlenmeyer flasks, glass stir rods, hot plate, filter paper, autoclave, incubator (37 °C), UV–Vis spectrophotometer (625 nm), Petri dishes, micropipettes with sterile tips, inoculating loop, sterile spreader, sterile forceps, sterile 6 mm paper discs, and a vernier caliper for measuring inhibition zones. The

materials used were fresh leaves of *Averrhoa bilimbi* Linn. and flowers of *Cananga odorata* (Lam.) Hook.f. & Thomson collected from Pontianak, West Kalimantan, sterile distilled water, clindamycin solution (300 mg/10 mL), Nutrient Broth (NB), Mueller–Hinton Agar (MHA), and a culture of *Cutibacterium acnes* as the test bacterium.

Preparation of Plant Infusions

Fresh *Averrhoa bilimbi* leaves and *Cananga odorata* flowers were washed under running water, drained, and cut into small pieces. Each plant material (100 g) was extracted separately using the infusion method by heating with distilled water at 90–95 °C for 15 min [14], [15]. The hot mixture was filtered while still warm, and the resulting filtrate was used directly as the undiluted infusion preparation. The combination treatments were then prepared by mixing the two undiluted infusions at ratios of 2:1, 1:2, and 1:1 (v/v). Sterile distilled water was used as the negative control, whereas clindamycin solution (300 mg/10 mL) served as the positive control.

Preparation of Bacterial Inoculum

A rejuvenated colony of *Cutibacterium acnes* was inoculated into 25 mL of Nutrient Broth and incubated until sufficient turbidity was achieved for antibacterial testing. Culture growth was monitored by measuring optical density at 625 nm using a UV–Vis spectrophotometer, and the suspension was used for antibacterial testing after reaching an OD of 0.8–1.0 [16]. Prior to inoculation, the bacterial suspension was homogenized to ensure a uniform inoculum.

Antibacterial Activity Assay

Antibacterial activity was evaluated using the disc diffusion method. Sterile paper discs with a diameter of 6 mm were immersed in each treatment solution for 30 min prior to application. For the positive control, 20 µL of clindamycin solution was applied to each sterile disc before placement on the agar surface. A 0.1 mL aliquot of the standardized *C. acnes* suspension was spread evenly onto the surface of Mueller–Hinton Agar using a sterile spreader. The impregnated discs were then aseptically placed on the inoculated agar surface and incubated at 37 °C for 24 and 48 h under anaerobic conditions.

Inhibition zones were measured after 24 and 48 h using a vernier caliper in four directions, namely vertical, horizontal, and two diagonal measurements, and the mean value was calculated using the following formula:

$$\text{Inhibition-zone diameter} = \frac{D_v + D_h + D_{g1} + D_{g2}}{4}$$

where D_v is the vertical diameter of the clear zone, $D_h$is the horizontal diameter, $D_{g1}$is the first diagonal diameter, and $D_{g2}$is the second diagonal diameter. In the present study, the reported inhibition-zone diameter referred to the total clear-zone diameter measured across the disc. Antibacterial activity was categorized according to Davis and Stout [17] as weak (<5 mm), moderate (5–10 mm), strong (10–20 mm), or very strong (≥20 mm).

Data Analysis

The inhibition-zone diameter data were analyzed using the Kruskal–Wallis test with IBM SPSS Statistics version 29.0. When the overall test showed statistical significance at $p < 0.05$, pairwise comparisons were performed using the Mann–Whitney test to identify differences between treatment groups. Data are presented as mean ± standard deviation (SD).

3. Results and Discussion

Inhibition Zone Profiles Against *Cutibacterium acnes*

The antibacterial activity of single and combination infusions of *Averrhoa bilimbi* leaves and *Cananga odorata* flowers against *Cutibacterium acnes* is presented in **Table 1**, with visual representations shown in **Figure 1** and **Figure 2**. Inhibition zones were observed in all infusion-treated groups at both 24 and 48 h of incubation, whereas the negative control did not produce any inhibition zone. By contrast, the positive control (clindamycin) produced the largest inhibition zone at both observation times.

Table 1. Mean Inhibition-Zone Diameters of Each Treatment Against *Cutibacterium acnes* After 24 and 48 Hours of Incubation

Treatment	24 h (mm)	48 h (mm)	Category
Negative control (sterile distilled water)	0.000 ± 0.0000[a]	0.000 ± 0.0000[a]	None
Positive control (clindamycin 300 mg)	10.800 ± 3.2696[b]	11.900 ± 3.3779[c]	Strong
Averrhoa bilimbi infusion	7.100 ± 0.0400[b]	6.133 ± 0.2309[b]	Moderate
Cananga odorata infusion	6.767 ± 0.4163[b]	6.167 ± 0.2887[b]	Moderate
Combination infusion 1 (*A. bilimbi* : *C. odorata* = 2:1)	6.900 ± 0.2000[b]	6.000 ± 0.0000[b]	Moderate
Combination infusion 2 (*A. bilimbi* : *C. odorata* = 1:2)	6.500 ± 0.7810[b]	6.400 ± 0.6928[b]	Moderate
Combination infusion 3 (*A. bilimbi* : *C. odorata* = 1:1)	7.533 ± 0.5132[b]	6.000 ± 0.0000[b]	Moderate

Note: *Values followed by different superscript letters within the same column indicate a significant difference at a = 0.05 based on the Mann–Whitney test. Data are presented as mean ± standard deviation.*

At 24 h, the positive control showed a mean inhibition-zone diameter of 10.800 ± 3.2696 mm. Among the infusion treatments, the largest mean inhibition zone was observed in combination infusion 3 (*A. bilimbi* : *C. odorata* = 1:1), with a diameter of 7.533 ± 0.5132 mm, followed by the single infusion of *A. bilimbi* leaves (7.100 ± 0.0400 mm), combination infusion 1 (6.900 ± 0.2000 mm), single infusion of *C. odorata* flowers (6.767 ± 0.4163 mm), and combination infusion 2 (6.500 ± 0.7810 mm). All infusion treatments were classified in the **moderate** category based on the Davis and Stout classification [17].

After 48 h of incubation, the positive control remained the treatment with the largest inhibition zone, increasing to 11.900 ± 3.3779 mm. In the infusion-treated groups, the inhibition zones ranged from 6.000 ± 0.0000 mm to 6.400 ± 0.6928 mm. The largest mean inhibition zone at this time point was observed in combination infusion 2 (*A. bilimbi* : *C. odorata* = 1:2), with a value of 6.400 ± 0.6928 mm, followed by the single infusion of *C. odorata* flowers (6.167 ± 0.2887 mm), the single infusion of *A. bilimbi* leaves (6.133 ± 0.2309 mm), and both combination infusion 1 and combination infusion 3, each with a mean inhibition-zone diameter of 6.000 ± 0.0000 mm. As at 24 h, all infusion treatments remained within the **moderate** inhibition category [17].

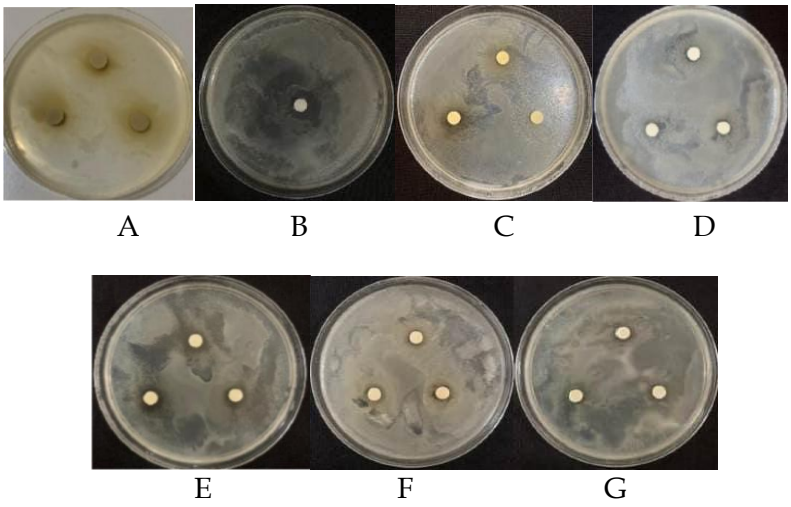

Figure 1. Inhibition zones produced by single and combination infusions against *Cutibacterium acnes* after 24 h of incubation. (A) Negative control (sterile distilled water); (B) Positive control (clindamycin); (C) *Averrhoa bilimbi* leaf infusion; (D) *Cananga odorata* flower infusion; (E) Combination infusion 1 (*A. bilimbi* : *C. odorata* = 2:1); (F) Combination infusion 2 (*A. bilimbi* : *C. odorata* = 1:2); (G) Combination infusion 3 (*A. bilimbi* : *C. odorata* = 1:1).

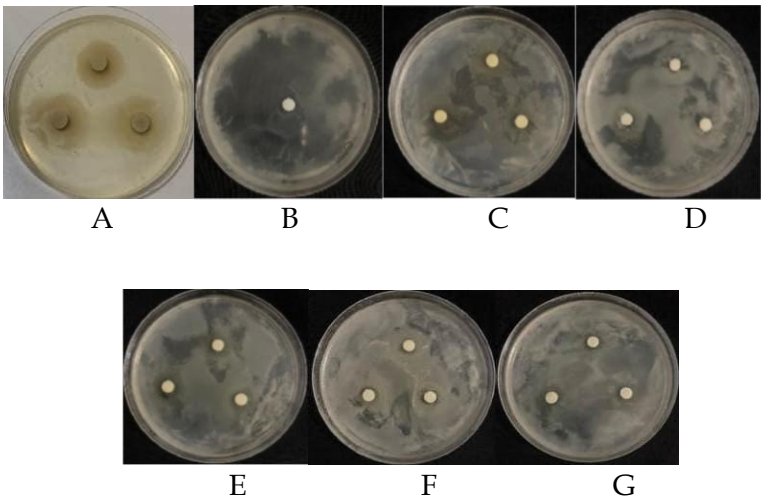

Figure 2. Inhibition zones produced by single and combination infusions against *Cutibacterium acnes* after 48 h of incubation. (A) Negative control (sterile distilled water); (B) Positive control (clindamycin); (C) *Averrhoa bilimbi* leaf infusion; (D) *Cananga odorata* flower infusion; (E) Combination infusion 1 (*A. bilimbi* : *C. odorata* = 2:1); (F) Combination infusion 2 (*A. bilimbi* : *C. odorata* = 1:2); (G) Combination infusion 3 (*A. bilimbi* : *C. odorata* = 1:1).

Overall, the descriptive findings indicate that both the single and combination infusions were able to inhibit the growth of *C. acnes* under the present disc diffusion conditions. However, the inhibition zones produced by the infusion treatments were consistently smaller than those of the positive control and remained within a relatively narrow range across the different infusion

groups. The visual appearances of the inhibition zones after 24 and 48 h are shown in **Figure 1** and **Figure 2**, respectively.

Statistical Comparison of Antibacterial Activity Among Treatment Groups

The statistical analysis showed that antibacterial activity differed significantly among treatment groups at both observation times. At 24 h of incubation, the Kruskal–Wallis test indicated a statistically significant overall difference in inhibition-zone diameters among the tested groups. Subsequent pairwise analysis using the Mann–Whitney test showed that all infusion-treated groups differed significantly from the negative control, confirming that both the single and combination infusions exerted antibacterial activity against *Cutibacterium acnes* under the present disc diffusion conditions. However, no statistically significant difference was detected between the infusion-treated groups and the positive control under the present sample size. This finding should be interpreted cautiously, because the absence of statistical significance does not indicate equivalence in antibacterial effectiveness.

At 48 h of incubation, the Kruskal–Wallis test again showed a statistically significant overall difference among treatment groups. Further Mann–Whitney analysis demonstrated that the negative control differed significantly from the positive control, and that both controls differed significantly from all infusion-treated groups. In contrast, no statistically significant differences were observed among the infusion treatments themselves, including the single infusions and the three combination infusions. These findings indicate that, although all infusion preparations exhibited measurable antibacterial activity, the statistical analysis did not demonstrate superiority of any particular infusion treatment over the others under the present experimental conditions.

Taken together, the statistical results support two main observations. First, all infusion treatments showed antibacterial activity relative to the negative control. Second, no statistically significant enhancement was detected among the different infusion formulations, including the combination groups, at either 24 or 48 h. Therefore, under the present sample size and disc diffusion conditions, the results support the presence of antibacterial activity but do not justify interpreting the combination treatments as statistically superior to the corresponding single infusions or as equivalent to clindamycin.

Phytochemical Basis and Possible Antibacterial Mechanisms

The antibacterial activity observed in the present study may be related to the phytochemical constituents previously reported in *Averrhoa bilimbi* leaves and *Cananga odorata* flowers. Belimbing wuluh leaf preparations have been reported to contain flavonoids, tannins, and saponins, all of which are associated with antibacterial activity [8], [19]. Similarly, kenanga flowers have been reported to contain flavonoids, tannins, saponins, and steroids [10], and previous studies have also demonstrated antibacterial activity of kenanga flower extracts against *Cutibacterium acnes* and other bacteria [11], [20]. These findings provide a plausible phytochemical basis for the inhibition zones produced by both the single and combination infusions in the present study.

Among these constituents, flavonoids are likely to play an important role in the inhibition of *C. acnes*. Flavonoids are widely recognized as antibacterial polyphenols that may interfere with bacterial survival through several mechanisms, including disruption of cytoplasmic membrane function, inhibition of nucleic acid synthesis, impairment of energy metabolism, and alteration of membrane permeability [21]–[23]. Because *C. acnes* is a Gram-positive bacterium, these mechanisms are particularly relevant, as flavonoids may interact directly with the phospholipid bilayer and weaken membrane integrity, thereby reducing bacterial viability [21], [22].

Tannins may further contribute to the observed antibacterial activity through their ability to precipitate proteins, chelate metal ions, interfere with peptidoglycan synthesis, and disrupt bacterial membrane integrity [24], [26]. These actions may impair structural stability and metabolic processes essential for bacterial survival. In addition, saponins are known to increase membrane permeability by interacting with lipid components of the bacterial cell envelope, which may facilitate leakage of intracellular contents and enhance susceptibility to other active compounds [27]. The presence of these metabolites in both plant materials suggests that the observed inhibition of *C. acnes* may result from the combined contribution of multiple phytochemical classes rather than from a single dominant constituent.

In kenanga flowers, the reported presence of steroids may also contribute to antibacterial activity, although their role is generally considered less prominent than that of flavonoids, tannins, and saponins [10]. Nevertheless, the coexistence of several bioactive compounds in both plant materials may support a multi-target antibacterial effect involving membrane disruption, increased permeability, interference with cell wall synthesis, and disturbance of bacterial metabolism. Accordingly, the antibacterial activity observed in the present study is biologically plausible based on the reported phytochemical profiles of *Averrhoa bilimbi* and *Cananga odorata*. However, because the present study did not include phytochemical quantification or compound isolation, these mechanisms should be interpreted as possible explanations rather than definitive proof of action.

Interpretation of the Combination Effect Under Disc Diffusion Conditions

Under the present disc diffusion conditions, the combination infusions of *Averrhoa bilimbi* leaves and *Cananga odorata* flowers did not demonstrate greater antibacterial activity than the corresponding single infusions. Although all combination treatments produced measurable inhibition zones against *Cutibacterium acnes*, their diameters remained within a relatively narrow range and did not show statistically significant enhancement over the single-infusion groups at either 24 or 48 h of incubation. These findings indicate that, within the limits of the current assay, no enhancement beyond the single infusions was observed.

In principle, the interaction between two antibacterial agents may be described as synergistic, antagonistic, or additive, depending on whether the combined effect is greater than, weaker than, or similar to the effect of the individual components [12], [28]. However, the present study did not employ specific interaction-testing methods such as checkerboard analysis with fractional inhibitory concentration (FIC) indexing or time-kill assays. Therefore, the current results should not be interpreted as sufficient evidence to formally classify the interaction between the two infusions. Instead, the findings should be limited to the observation that the combination treatments showed antibacterial activity, but no statistically demonstrable enhancement over the single infusions was detected under the present disc diffusion conditions.

This interpretation is also important in relation to previous studies reporting beneficial effects of combining plant-based preparations. For example, Otieno et al. [12] described the potential of multi-plant combinations to improve antibacterial inhibition, while Budiarti et al. [13] reported that a combination of belimbing wuluh and kenanga preparations was effective against several pathogenic bacteria. Nevertheless, differences in plant part, extraction method, bacterial target, and assay design may influence the apparent outcome of combination testing. Accordingly, the present findings should be interpreted conservatively: the combination infusions were active against *C. acnes*, but

the available disc diffusion data do not support a formal conclusion that the interaction was additive or synergistic.

Time-Dependent Changes in Inhibition Zones and Stability Considerations

A comparison of inhibition-zone diameters at 24 and 48 h showed a general decline in antibacterial activity among the infusion-treated groups over time. As shown in **Table 1**, the inhibition zones of all infusion preparations tended to decrease at 48 h compared with those observed at 24 h. In contrast, the positive control (clindamycin) maintained, and numerically even increased, its inhibition-zone diameter over the same period. This pattern suggests that the antibacterial activity of the plant infusions was less persistent than that of the antibiotic control under the present disc diffusion conditions.

One possible explanation for the reduced inhibition zones at 48 h is the instability of active compounds present in the infusions. The antibacterial activity of belimbing wuluh and kenanga preparations is likely associated with phytochemicals such as flavonoids, tannins, saponins, and volatile components, some of which may be susceptible to degradation, oxidation, evaporation, or other physicochemical changes during prolonged incubation [18], [29]. Because infusion preparations are water-based and are generally considered less stable than more concentrated or solvent-based extracts, a gradual reduction in active compound availability may contribute to the decline in antibacterial performance over time [18], [30], [31].

However, the reduction in inhibition-zone diameter should not be interpreted too narrowly. In disc diffusion assays, zone changes over time may reflect not only reduced persistence of activity, but also bacterial regrowth, diffusion dynamics, compound instability, and measurement variability. Therefore, although the smaller inhibition zones at 48 h may suggest reduced persistence of antibacterial activity in the infusion preparations, disc diffusion alone is insufficient to confirm a bacteriostatic mechanism. This interpretation is more consistent with the reviewer's concern that time-dependent zone reduction should be discussed cautiously rather than used as definitive evidence of bacteriostatic action.

The behavior of the positive control may also support this interpretation. Clindamycin showed the largest inhibition zone at both observation times and did not exhibit the decline seen in the plant infusions. This may be related to its well-established antibacterial mechanism and more stable performance under the test conditions [32]. Nevertheless, the numerical increase in clindamycin zone diameter over time should also be interpreted cautiously, because diffusion characteristics and ongoing interaction with the agar medium may influence zone size independently of intrinsic antibacterial potency.

Previous studies on plant infusion or decoction preparations have likewise suggested that antibacterial activity may decrease with storage time or prolonged exposure because of degradation of active constituents [29]. Studies such as those by Budiarti et al. [13] and Lumbantoruan [33] may support the broader observation that plant-based preparations can inhibit bacterial growth without necessarily demonstrating durable or bactericidal effects; however, these comparisons should not be taken as direct proof that the present infusions act bacteriostatically. Accordingly, the current findings are best interpreted as showing a time-dependent reduction in inhibition zones, which may reflect reduced persistence of antibacterial activity, but require further confirmation through MIC/MBC determination, time-kill analysis, and stability testing.

Study Limitations

This study has several limitations that should be considered when interpreting the findings. First, antibacterial activity was evaluated only using the disc diffusion method, which provides qualitative to semi-quantitative information and does not allow definitive determination of minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC). Second, the present study did not assess the interaction between the two infusions using specific combination-testing methods such as checkerboard analysis with fractional inhibitory concentration (FIC) indexing or time-kill assays; therefore, the nature of the interaction between *Averrhoa bilimbi* and *Cananga odorata* infusions cannot be formally classified. Third, the study did not identify or quantify the active phytochemical constituents responsible for the observed antibacterial activity, so the contribution of individual compounds remains uncertain. Fourth, the use of infusion preparations may have affected the stability of bioactive compounds because water-based extracts are generally more susceptible to degradation during storage and prolonged incubation. In addition, the experiment was conducted only under in vitro conditions with a limited number of replicates, which may restrict the generalizability and statistical robustness of the findings. Therefore, further studies incorporating MIC/MBC determination, checkerboard or time-kill analysis, phytochemical profiling, stability testing, and broader experimental replication are needed to strengthen the evidence for the antibacterial potential of these plant infusions against *Cutibacterium acnes*.

4. Conclusion

The infusions of *Averrhoa bilimbi* leaves and *Cananga odorata* flowers showed antibacterial activity against *Cutibacterium acnes* in the moderate category under disc diffusion conditions. The combination infusions did not show statistically detectable enhancement over the single infusions, and the reduction in inhibition zones at 48 h should not be interpreted as definitive evidence of a bacteriostatic effect. Further studies using MIC/MBC, checkerboard or FIC, time-kill, stability, and phytochemical analyses are required to confirm the antibacterial characteristics and interaction of these infusions.

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Conflict of Interest:

The authors declare no conflict of interest related to this study.

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