

# ANTIBACTERIAL ACTIVITY OF DR. RIDZ ANTI-ACNE NANOSERUM® AGAINST SKIN BACTERIA THAT CAUSE ACNE VULGARIS

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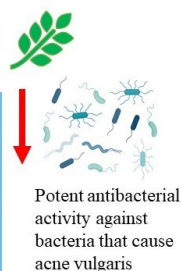
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## Graphical abstract

Plant-based product,  
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## Abstract

*Cutibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* are commonly associated with the pathogenesis of acne vulgaris. Skincare products that contain natural ingredients have become a trend to treat acne. Dr.Ridz Anti-Acne Nanoserum® product was formulated with the extract of *Cassia alata* leaf and *Piper betle*. The aim of this study was to determine the antibacterial activity of this formulated product against *C. acnes*, *S. epidermidis*, and *S. aureus*. The minimum inhibitory concentration (MIC) value and the minimum bactericidal concentration (MBC) were determined using a broth microdilution and a streak plate method, respectively. The rate of killing by this product was tested using time-kill assay (TKA) at 0.5x MIC, 1x MIC, and 2x MIC with incubation periods of 2, 4, 6, 8, 10, 12, and 24 hours. The MIC values showed that this product inhibited *S. epidermidis* and *S. aureus* better than *C. acnes*. From the calculation of the MBC/MIC ratio, this product showed bactericidal effects against all tested bacteria. The time-kill studies showed that the killing effect of this product was concentration-dependent, and the highest antibacterial activity was observed at the concentration of 2x MIC against all tested bacteria. In conclusion, Dr.Ridz Anti-Acne Nanoserum® exhibits the best antibacterial activities against *S. epidermidis* and showed a bactericidal effect towards all tested bacteria.

**Keywords:** Acne vulgaris, antibacterial activity, acne pathogens, *Piper betle*, *Cassia alata*

## Abstrak

*Cutibacterium acnes*, *Staphylococcus epidermidis*, dan *Staphylococcus aureus* selalunya dikaitkan dengan patogenesis acne vulgaris. Produk penjagaan kulit yang mengandungi bahan semulajadi telah menjadi trend yang digunakan untuk merawat acne. Dr.Ridz Anti-Acne Nanoserum® telah diformulasikan menggunakan ekstrak daun *Cassia alata* dan *Piper betle*. Tujuan kajian ini adalah untuk menentukan aktiviti antibakterial produk formulasi ini terhadap *C. acnes*, *S. epidermidis*, dan *S. aureus*. Nilai kepekatan perencat minimum (MIC) dan kepekatan bakterisidal minimum (MBC) telah ditentukan menggunakan pencairan mikro kaldu dan kaedah piring coretan. Kadar pembunuhan bakteria ditentukan menggunakan asai "time-kill" pada

0.5X MIC, 1X MIC, dan 2X MIC pada pengeraman 2, 4, 6, 8, 10, 12, dan 24 jam. Nilai MIC menunjukkan produk ini merencat *S. aureus* lebih baik berbanding *C. acnes*. Pengiraan nisbah MBC/MIC menunjukkan produk ini mempunyai kesan bakterisidal terhadap semua bakteria yang diuji. Ujian "time-kill" menunjukkan kesan bakterisidal adalah bergantung pada kepekatan dan nilai tertinggi adalah pada 2X MIC. Kesimpulannya, Dr.Ridz Anti-Acne Nanoserum® menunjukkan kesan antibakterial terbaik terhadap *S. epidermidis* dan antibakterial terhadap semua bakteria yang diuji.

**Kata kunci:** Acne vulgaris, aktiviti antibakterial, patogen acne vulgaris, *Piper betle*, *Cassia alata*

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## 1.0 INTRODUCTION

Acne vulgaris is a prevalent skin disorder that predominantly affects teenagers and young adults who undergo puberty due to the high production of sebum. The growth of bacteria that cause acne vulgaris will be increased, with an unbalanced diet and hormones which are susceptible to this disease [1].

[1] Numerous factors lead to this disease, including an unbalanced diet, genetic factors, and hormonal imbalance. Four major factors can stimulate the pathogenesis of this disease, including increased sebum production, excessive keratin production that leads to the blockage of the pilosebaceous duct, imbalance of the normal flora especially *C. acnes*, and skin inflammation [2]. Several bacteria species have been reported to be associated with acne problems, which include *C. acnes*, *S. epidermidis*, and *S. aureus* [3].

*C. acnes* (formerly known as *Propionibacterium acnes*) is one of the normal floras that is widely found on the surface of human skin. *C. acnes* is an anaerobic, aerotolerant bacteria that prefers to live in the pores of the human skin's surface. This is due to the presence of sebum in skin pores, which is a crucial substrate for the growth of *C. acnes* [4]. As reported by Behzadi *et al.* [5], acne vulgaris is one of the infections caused by *C. acnes* imbalance.

*S. epidermidis* is another normal flora frequently found on the surface of the skin and belongs to the coagulase-negative staphylococci (CoNS) group [6]. These bacteria are often referred to as incidental pathogens because they can cause harm to the host unintentionally. *S. epidermidis* generates biofilms that can create anaerobic conditions and hence provide a favourable environment for *C. acnes* colonisation. As a result, the concentration of *C. acnes* on the skin surface rises and inevitably leads to the pathogenesis of acne vulgaris [7].

Besides, *S. aureus* is one of the major nosocomial pathogens in causing soft tissue and skin infections. These bacteria are often associated with the formation of abscesses and furuncles on the surface of the skin [8]. Resistance of *S. aureus* against multiple antibiotics leads to difficulties in treating infections caused by this bacterium [9].

Nowadays, the usage of skincare products has been increasingly popular in recent years, particularly among youngsters. Most people use these products to treat acne and other skin disorders. To retain their durability, smell, and function, skincare products are often manufactured with a variety of synthetic compounds and heavy metals [10]. Most of these compounds can harm the skin, especially people who have sensitive skin. As a result, consumers currently prefer products made from natural substances, which have fewer side effects than synthetic ones [11].

The benefits of medicinal plants have been acknowledged and widely applied in the food, cosmetics, and pharmaceutical industries. This is because most herbal plants are proven to contain active ingredients that are capable of treating various skin problems and they possess good safety profiles [12, 13]. Medicinal plant advantages have long been recognized and widely used in the food, cosmetics, and pharmaceutical industries. For instance, multiple studies suggest that betel leaves have strong antibacterial and antifungal effects, as well as a favorable safety profile [14, 15, 16].

Therefore, Dr. Ridz's Anti-Acne Nanoserum® product enriched with *Cassia alata* leaves extract and *Piper betle* was tested to evaluate the antibacterial activity of this product against *C. acnes*, *S. epidermidis*, and *S. aureus*. This skincare product consists of 80% of leaf extracts that have been recognized to exhibit strong antimicrobial properties and hence is expected to have good antibacterial activity.

## 2.0 METHODOLOGY

### 2.1 Test Material

Dr. Ridz Anti-Acne Nanoserum® (Estika Research Centre, Terengganu, Malaysia), a commercial skincare product was evaluated. This product was formulated with the leaf extract of *Cassia alata*, *Piper betle*, seaweed (Personal Formula Resources (M)Sdn. Bhd, Malaysia), and tea tree (Rakesh Sandal Industry, India). Vancomycin was used as a positive control for *C. acnes*, *S. epidermidis*, and *S. aureus* as it is usually

used as the last resort for treatment in bacterial infection [17] and it was provided by the Faculty of Health Sciences, the National University of Malaysia.

## 2.2 Test Organisms

To conduct this study, a total of three strains of bacteria that cause acne, which are *C. acnes*, *S. epidermidis*, and *S. aureus* were used. Reference strains of *C. acnes* ATCC 6919 and *S. aureus* ATCC 25923 were obtained from Management and Science University (MSU), Shah Alam Campus, Selangor. Meanwhile, *S. epidermidis* was a clinical isolate from Hospital Canselor Tuanku Muhriz of UKM (HCTM), Cheras, Malaysia.

## 2.3 Determination of Minimal Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) value of Dr. Ridz Anti-Acne Nanoserum® was determined using the broth microdilution method described previously [18]. Depending on the bacterial strains tested, each 96-well microtiter plate was filled with 50 µl broth. Mueller-Hinton broth (MHB) was used to test for *S. epidermidis* and *S. aureus*, while Trypticase Soy broth (TSB) was used to test for *C. acnes*. A 50 µl tested product was loaded in the first well of the 96-well microtiter. Then, from the total aliquots inside the well, 50 µl was moved to the next well, and so on until the tenth well. This procedure was used to serially dilute the product from 100% concentration to 50% concentration until the final concentration of 0.097%. Next, 50 µl of bacterial inoculum ( $1.0 \times 10^8$  CFU/mL) was added to the well that contained the tested product. For *C. acnes*, the plate was incubated anaerobically at 37°C for 24 h. Meanwhile, the plate was incubated at 37°C at aerobic conditions for 18 hours for *S. epidermidis* and *S. aureus*. After incubation, the TTC reagent (2,3,5-triphenyl tetrazolium chloride) was added to each well containing the test material. The antibacterial activity of the product was assessed by observing the color changes in the test wells. The absence of color changes indicated that bacteria were not present, or that the number of bacteria present in the well was minimal. Meanwhile, the color change from colourless to reddish suggests that bacterial activity is still present inside the well [19]. To ensure that the results were valid, the procedure was repeated three times for each organism.

## 2.4 Determination of Minimal Bactericidal Concentration (MBC)

The streak plate method was performed to determine the value of minimum bactericidal concentration (MBC) on *C. acnes*, *S. epidermidis*, and *S. aureus*. A total of 50 µl from wells that did not show any color changes were cultured onto different agar. *C. acnes* was cultured on Soy Trypticase agar (TSA), whereas *S. epidermidis* and *S. aureus* were

cultured on Mueller Hinton agar (MHA). The plate was incubated at 37°C under anaerobic conditions for *C. Acnes* and at 37°C aerobically for *S. epidermidis* and *S. aureus*. The procedure was repeated three times to ensure that the results were valid.

## 2.5 Time-Kill Kinetics Assay

Time-kill assay on Dr. Ridz Anti-Acne Nanoserum® against *C. acnes*, *S. epidermidis*, and *S. aureus* was performed according to Clinical Laboratory Standards Institute [20] and Appiah et al. [21]. The product was evaluated at concentrations of 0.5x MIC, 1x MIC, and 2x MIC, whereas vancomycin was used as a positive control at a concentration of 1x MIC. For growth control, bacterial inoculum ( $1.0 \times 10^8$  CFU/mL) was used. Then, 20 µl aliquots from the conical flask of each treatment group were taken at time intervals of 0, 2, 4, 6, 8, 10, 12, and 24 h. Time points from 0-24 hours were chosen because evaluation of the product against the bacteria tested could be conducted at short-term (0-12 hr) and long-term duration (12-24 hr). Tenfold serial dilution of the aliquot was conducted from  $10^{-1}$  to  $10^{-10}$ . 100 µl of the serial dilution aliquot was taken and distributed over the plate using the spread plate method. Afterward, the plate was incubated at 37°C under anaerobic conditions for *C. acnes* and normal conditions for *S. epidermidis* and *S. aureus*. The number of viable cells was counted after incubation, and the CFU/ml was determined. The procedure was performed in triplicate. Finally, the time-kill kinetics curve was plotted, and the data was analyzed.

## 3.0 RESULTS AND DISCUSSION

### 3.1 MIC, MBC, and MBC/MIC Ratio of Dr. Ridz Anti-Acne Nanoserum®

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of Dr. Ridz Anti-Acne Nanoserum®. Table 1 summarises the value MIC, MBC, and MBC/MIC ratio of Dr. Ridz Anti-Acne Nanoserum® against *C. acnes*, *S. epidermidis*, and *S. aureus*. The MIC value of this product against *C. acnes* was 25%. Meanwhile, *S. epidermidis* and *S. aureus* have the same MIC value which was 12.5%.

**Table 1** The value of MIC, MBC, and MBC/MIC Ratio of Dr. Ridz Anti-Acne Nanoserum® product against bacterial strains tested

Bacterial strains	MIC (%)	MBC (%)	MBC/MIC Ratio
<i>Cutibacterium acnes</i>	25	50	2
<i>Staphylococcus epidermidis</i>	12.5	25	2
<i>Staphylococcus aureus</i>	12.5	50	4

Determination of the minimum bactericidal concentration (MBC) on Dr. Ridz Anti-Acne Nanoserum® was carried out using the streak plate method. The MBC values for the tested product against *C. acnes* and *S. aureus* were similar at 50%. Meanwhile, the MBC value of the tested product against *S. epidermidis* was 25%. MBC/MIC ratio value is 4 or less, the test substance has a bactericidal effect. MBC/MIC ratio value shows more than 4, the test substance has a bacteriostatic effect [22]. The MBC/MIC ratio is calculated based on the MBC and MIC values obtained. The findings revealed that the tested product exhibited bactericidal effects against all bacterial strains. Dr. Ridz Anti-Acne Nanoserum® demonstrated good bactericidal effect against *C. acnes* and *S. epidermidis*, (Table 1), with a low MBC/MIC ratio of 2. For *S. aureus*, the MBC/MIC ratio reading obtained was 4, indicating bactericidal effects against *S. aureus*.

The inhibitory effect on the 3 tested organisms suggests a relation with the main ingredients used in Dr. Ridz Anti-Acne Nanoserum® including the extract of *Cassia alata* leaves and *Piper betle*. Studies conducted previously [23, 24] indicated that *C. alata* leaf extract has a better inhibitory effect on *S. Aureus* (0.39 mg/mL) than *C. Acnes* (0.625 mg/mL). *C. alata* extract, on the other hand, was found to exhibit similar antibacterial properties on *S. epidermidis* (inhibition zone: 20 mm) and *S. aureus* (inhibition zone: 16 mm), as described previously [25]. This demonstrated that the findings of antibacterial activity in Dr. Ridz Anti-Acne Nanoserum® is consistent to previous studies and exhibits similarities in inhibitory effects against the organisms tested. In comparison to *C. acnes*, the extract of *C. alata* leaves, which is one of the key constituents in test products, have a good inhibitory effect on *S. epidermidis* and *S. aureus*.

Dr. Ridz Anti-Acne Nanoserum® killed *C. acnes* and *S. aureus* at 50% concentration. Meanwhile, at a lesser concentration of 25%, this substance showed bactericidal effect on *S. epidermidis*. This is consistent with previous research that investigated the antibacterial activity of *C. alata* and *P. betle* leaf extracts conducted alone per single plant on acne-causing bacteria that showed similarities in bactericidal effects. The MBC value determined previously [23, 24] indicated that the *C. alata* leaf extract had a bactericidal effect against *C. acnes*

(MBC: 1.25 mg/mL) at a lower concentration than *S. aureus* (5.21 mg/mL). In addition, there was a study to test the antibacterial activity of creams formulated with *P. betle* leaf extract using disk diffusin assay and the results showed that the antibacterial effect against *C. acnes* occurred at lower concentrations (zone of inhibition) when compared to *S. aureus* [26]. Meanwhile, a study conducted by [27] showed that *P. betle* extract using disk diffusion assay showed a better potential in inhibiting and killing *S. Epidermidis* (based on zone of inhibition assay) compared to *S. aureus*. Based on these previous studies, Dr. Ridz Anti-Acne Nanoserum® product exhibited the same bactericidal effect as the study stated, where the MBC value obtained was lower against *S. epidermidis* followed by *C. acnes* and subsequently *S. aureus* which showed the weakest bactericidal effect.

MBC/MIC ratio that is 4 and below indicates the test material showed a bactericidal effect and if the MBC/MIC ratio shows a reading above 4 indicates the test material showed bacteriostatic effects [22]. Hence, Dr. Ridz Anti-Acne Nanoserum® demonstrated bactericidal effect against all microbes tested in this study. The MBC/MIC ratio for *C. acnes* and *S. epidermidis* was 2, indicating that this product had a good bactericidal effect against both organisms. When compared to *C. acnes* and *S. epidermidis*, the MBC/MIC ratio for *S. aureus* is 4, indicating that the tested product had a slightly weaker bactericidal effect. The results of the MBC/MIC ratio are connected with the MBC values obtained, which showed that the tested product could kill *C. acnes* and *S. epidermidis* more effectively than *S. aureus*. Overall, the MBC values showed that Dr. Ridz Anti-Acne Nanoserum® had good bactericidal effect on *S. epidermidis*, which could be killed at lower concentrations than *C. acnes* and *S. aureus*. However, based on the MBC/MIC ratio acquired, this product had a good bactericidal effect against *C. acnes*, and *S. aureus* had a bactericidal effect as well, though significantly weaker than the other studied organisms.

Dr. Ridz Anti-Acne Nanoserum® was formulated with two herbs that are famous in Southeast Asia, namely 'Dau Gelenggang' (*Cassia alata* leaf) and 'Sireh' (*Piper betel*). Khan et al. [25] reported that *C. alata* has extensive antibacterial activities and each part of the plant including leaves, flowers, and stems has its medicinal benefits. This statement is supported by Owoyale et al. [13] which stated that *C. alata* leaf extract showed good antibacterial and antifungal activities. Furthermore, *P. betle* is often applied in the pharmaceutical and food industries because this herb has extensive antimicrobial activities and also displays a good safety profile [14]. It is suggested that the combination of two herbal plants with good antimicrobial properties gave good antibacterial activities of Dr. Ridz Anti-Acne Nanoserum® against *C. acnes*, *S. epidermidis*, and *S. aureus*.

In addition, there are other natural ingredients formulated in this product which include seaweed and tea tree. Seaweed is often associated with

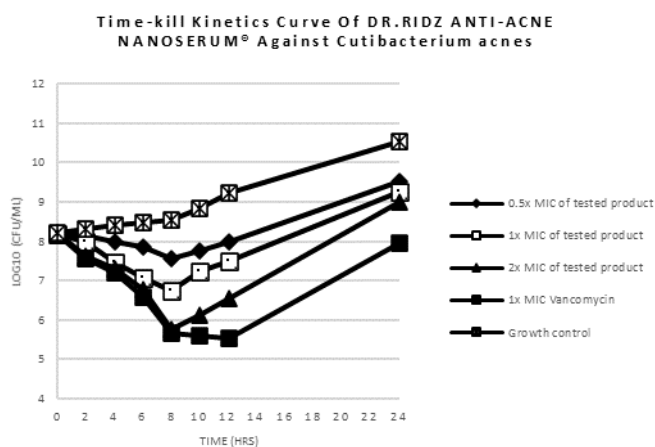
natural ingredients that can slow down the aging process and treat the problem of hyperpigmentation on the skin surfaces [28]. Furthermore, some studies reported that seaweed also shows good antimicrobial properties. Based on a study conducted by Choi *et al.* [29], seaweed does show strong antibacterial activity against *C. acnes* and also has anti-inflammatory properties. Seaweed has also been reported to have an effective antibacterial effect against *S. aureus* [30]. Meanwhile, studies on the antimicrobial effects of tea trees have also been extensively conducted. A study conducted previously [31] has reported that the oil produced from tea trees exhibits good antibacterial activity against *C. acnes* and can treat wounds caused by acne vulgaris infection. Meanwhile, Esmael *et al.* [32] stated that the tea tree is a good agent to be used as an antibiotic to treat acne vulgaris that occurs due to *C. acnes*, *S. epidermidis*, and *S. aureus*. Thus, this proves that the use of seaweed and tea tree in Dr. Ridz Anti-Acne Nanoserum® are not only able to bring functions such as increasing skin elasticity, slowing the aging process, and treating hyperpigmentation but these two natural ingredients are also believed to help in further enhancing the antimicrobial effect of this product.

Next, the product also uses several synthetic materials that act as stabilizers, emulsifiers, pH regulators, and preservatives. Ethylhexylglycerin (EHG), which serves as a preservative for this product, is one of the synthetic compounds that has antibacterial capabilities and is only allowed at less than 1% in cosmetic product products [33]. Our product uses 0.2% of EHG. The major purpose of preservatives in cosmetics is to ensure the antimicrobial effectiveness of that particular product [34]. According to Lawan *et al.* [35], EHG was reported to have antimicrobial activity against *S. aureus*, *P. aeruginosa*, *E. coli*, and *C. albicans*. At a concentration of 1.5%, this substance was able to inhibit the growth of these tested organisms and lasted up to one day. As a result, it is possible that EHG also directly helps Dr. Ridz Anti-Acne Nanoserum® products to show antibacterial effects, and further study is warranted. In addition, one of the stabilizing ingredients has also been formulated into the Dr. Ridz Anti-Acne Nanoserum® product and the ingredient is Phenoxyethanol (PE), which is used at a concentration of 0.8% in our product. The allowable value of PE in product formulation is less than 1% [36]. Several studies report that PE has a synergistic effect if synthetic preservatives are combined in a product. As reported by [33], synergistic effects resulting from the combination of PE and EHG exhibit better antimicrobial activities. In addition, Herman [37] reported that the combination of plant extracts and synthetic preservatives can improve the antimicrobial properties of a product. Therefore, the synergistic effect that occurs between natural products and synthetic materials used as stabilizers or preservatives, plays a role in enhancing the ability of Dr. Ridz Anti-

Acne Nanoserum® as a good antibacterial skincare product.

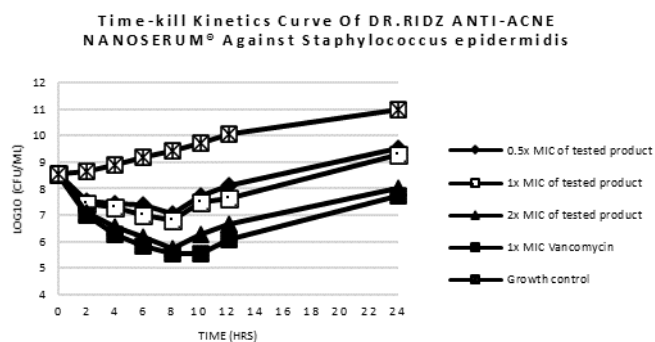
#### 4.2 Time-Kill Kinetics of Dr. Ridz Anti-Acne Nanoserum®

The time-kill kinetics profile of Dr. Ridz Anti-Acne Nanoserum® against *C. acnes* is shown in Figure 1. The concentrations of the product tested include 0.5x MIC, 1x MIC, and 2x MIC. A gradual decrease in the number of viable cells over each concentration tested could be observed and showed an increment at the eighth hour. The number of viable cells decreased the most at 2x MIC of the product ( $2.45 \log_{10} \text{CFU/ml}$ ), followed by 1x MIC ( $1.46 \log_{10} \text{CFU/ml}$ ) and 0.5x MIC ( $0.63 \log_{10} \text{CFU/ml}$ ).



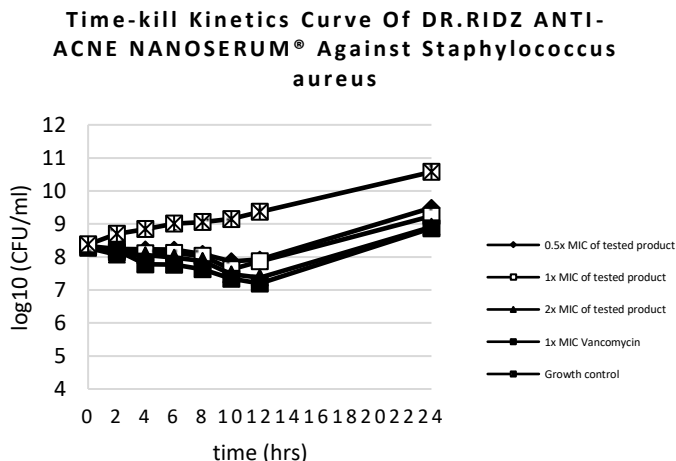
**Figure 1** Time-kill kinetics curve of Dr. Ridz Anti Acne Nanoserum® against *C. acnes*

Figure 2 depicts the profile of time-kill kinetics of Dr. Ridz Anti-Acne Nanoserum® against *S. epidermidis* at concentrations of 0.5x MIC, 1x MIC, and 2x MIC. The time-kill kinetics curve displayed a decrease in the number of viable cells with time for each concentration tested, with an increase after the eighth hour. The reduction in the number of CFU/ml at 2x MIC of the tested product was  $2.82 \log_{10}(\text{CFU/ml})$  which was the highest when compared to 1x MIC ( $1.72 \log_{10} \text{CFU/ml}$ ) and followed by 0.5x MIC ( $1.49 \log_{10} \text{CFU/ml}$ ).



**Figure 2** Time-kill kinetics curve of Dr. Ridz Anti-Acne Nanoserum® against *S. epidermidis*

Figure 3 illustrates the time-kill kinetics profile for *S. aureus* treated with Dr. Ridz Anti-Acne Nanoserum® at 0.5x MIC, 1x MIC, and 2x MIC concentrations. Each concentration tested showed a gradual decrease in the number of viable cells. At the tenth hour, a rise in bacterial activity was observed at concentrations of 0.5x MIC and 1x MIC. Whereas a 2x MIC concentration showed an increase in the number of viable cells at the twelfth hour. The decrease in the number of CFU/ml at 2x MIC of the tested product was the highest at 0.80 log<sub>10</sub>(CFU/ml), followed by 1x MIC (0.33 log<sub>10</sub> CFU/ml) and 0.5x MIC (0.24 log<sub>10</sub> CFU/ml).



**Figure 3** Time-kill kinetics curve of Dr. Ridz Anti-Acne Nanoserum® against *S. epidermidis*

Time-kill kinetics assays were performed to study and understand the interactions that occur between antimicrobial agents and organisms tested. These assays are often conducted to test whether the antimicrobial activity of an agent or tested product is concentration-dependent or time-dependent [38]. When *C. Acnes* were treated with Dr. Ridz Anti-Acne Nanoserum®, the time-kill curve indicated a

reduction in the number of viable cells up to the eighth hour, and then an increase in viable cells. This shows that the skincare products had an antibacterial effect that lasted for up to eight hours. Next, a decrease in the number of viable cells can be seen in the time-kill curve of this product against *S. epidermidis*. The number of viable cells began to rise after the eighth hour. This suggests that this product has the same antibacterial effect as *C. acnes*, with antibacterial activity lasting up to 8 hours. Meanwhile, the time-kill kinetics assay of Dr. Ridz Anti-Acne Nanoserum® against *S. aureus* differs from *C. acnes* and *S. epidermidis* time-kill curves. The number of viable cells decreased up to the tenth hour in the time-kill curve of *S. aureus* at concentrations of 0.5x MIC and 1x MIC, with an increase in the bacterial colony after that. At the twelfth hour, 2x MIC concentration showed an increase in bacterial activity. This demonstrates that this product has antibacterial activity against *S. aureus* that can last for about 10 to 12 hours in a concentration-dependent manner.

Time-kill assay analysis revealed that Dr. Ridz Anti-Acne Nanoserum® demonstrated good antibacterial activity against *C. acnes*, *S. epidermidis*, and *S. aureus*. The bacterial killing effect of this product was concentration-dependent, according to the time-kill curves of all organisms tested. The product with 2x MIC concentration demonstrated the most reduction in viable cells tested because 2x MIC had more active ingredients than the concentration of 0.5x MIC and 1x MIC. The result indicates that at its original concentration, Dr. Ridz Anti-Acne Nanoserum® is expected to have greater antibacterial activity, and thus the product has good potential as a skincare product with an antibacterial effect.

Consumers of skincare and beauty products prefer eco-friendly products that contain natural compounds derived from herbal plants than synthetic-based products. A survey conducted previously [39] showed that 87.1% of cosmetic product users (out of 94%) believed that synthetic chemical-based products can cause adverse effects. Meanwhile, only 6% of consumers believe that products made with natural ingredients can have negative side effects. One question arose as a result of the survey: Is it true that goods produced with these natural ingredients have no adverse effects? Some of the disadvantages of natural products includes contact dermatitis and urticaria [40]. Calendula, aloe vera, arnica, chamomile, and other natural products have been linked to this condition [41]. Furthermore, natural substances like licorice and fenugreek have been linked to hormone abnormalities, which can be harmful to consumers if not treated properly [42]. Even though these negative effects have been shown in multiple studies, most consumers still prefer natural-based products. This is because, according to a previous study [3], natural substances have fewer and generally milder negative effects than products containing synthetic compounds. Parabens, formaldehyde, mercury,

nickel, lead, Butylated Hydroxytoluene (BHT), Diethanolamine (DEA), and other synthetic chemicals have been linked to hazardous side effects [43]. To the best of our knowledge, the ingredients in our product are not exposed to those harmful chemicals. Many countries have banned or restricted the use of most of these synthetic chemicals because they are detrimental to human health, including increasing the risk of cancer development.

In contrast to the reported adverse effects, products formulated with natural ingredients seem to have many advantages and are reported to be very effective in medicine and cosmetics. Dr. Ridz Anti-Acne Nanoserum®, which is packed with natural ingredients, is predicted to be one of the commercial antibacterial skincare products that can assist consumers in resolving their skin issues, particularly acne vulgaris.

#### 4.0 CONCLUSION

Dr. Ridz Anti-Acne Nanoserum® can inhibit the growth of *C. acnes* at a concentration of 25%. At the 12.5% concentration, this product shows an inhibitory effect on *S. epidermidis* and *S. aureus*. This implies that, when compared to *C. acnes*, this product is more effective at inhibiting the growth of *S. epidermidis* and *S. aureus*. The product showed a bactericidal effect against *C. acnes*, *S. epidermidis*, and *S. aureus*. At 25% concentration, this product is capable of killing *S. epidermidis*. Meanwhile, this product showed a bactericidal effect against *C. acnes* and *S. aureus* at 50% concentration. The number of viable cells also decreased the most for *S. epidermidis* and *C. acnes*. As for the time-kill assay results, the highest decrease in the number of colonies for *C. acnes*, *S. epidermidis*, and *S. aureus* were 2.45, 2.82, and 0.80 log<sub>10</sub> CFU/ml, respectively. Dr. Ridz Anti-Acne Nanoserum® product also exhibited concentration-dependent antibacterial activity against *C. acnes*, *S. epidermidis*, and *S. aureus*. With this, Dr. Ridz Anti-Acne Nanoserum® is believed to have the potential to be one of the skincare products that can aid patients with acne vulgaris by exerting outstanding antibacterial properties.

#### Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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