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ANTIBACTERIAL ACTIVITY OF DADANGKAK ROOT EXTRACT (*Hydrolea spinosa L.*) AGAINST *Propionibacterium acnes* BACTERIA

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Abstract

Women are prone to acne and they often treat it with antibiotics. However, there is a negative impact from the use of antibiotics, namely resistance and the incidence of antibiotic resistance against *Propionibacterium acnes* in Indonesia is quite high. This is the reason for the use of compounds from the Dadangkak plant (*Hydrolea spinosa L.*) which are often used by people in South Kalimantan as treatment, especially in the root part which will be used as an antibacterial in *Propionibacterium acnes*. This study aims to determine the antibacterial activity of Dadangkak root extract against *Propionibacterium acnes* using the diffusion method and the dilution method to determine the value of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract. Testing the antibacterial activity of the extract of Dadangkak root (*Hydrolea spinosa L.*) against the bacteria *Propionibacterium acnes* using the disc diffusion method as a screening test and the dilution method for testing MIC and MBC with concentrations of 20%, 40%, 60%, 80%, and 100%. 1% Clindamycin was used as a positive control and DMSO was used as a negative control. The results showed that the extract of Dadangkak root (*Hydrolea spinosa L.*) had antibacterial activity which was indicated by the presence of a clear area around the disc and the MIC value at a concentration of 60% (0.06 g/ml) but no MBC value available. Based on the results of the research, it can be concluded that the extract of dadangak root has the potential as an antibacterial against bacteria *Propionibacterium acnes* with a Minimum Inhibitory Concentration value of 0,06 g/ml (*p value* in *Kruskall Wallis* = 0,018 and *p value* in *Post Hoc Mann-Whitney* test = 0,025)

Keywords: Antibacterial, Dadangkak Root Extract (*Hydrolea spinosa L.*), *Propionibacterium acnes*

1. Introduction

Women often experience facial problems such as acne. It is an abnormal skin condition caused by an oil gland disorder so that oil production on the face becomes excessive which results in blockages in the hair follicle channels and facial skin pores (Mumpuni and Wulandari, 2010).. Acne is a problem that often occurs in various ages and circles because it can lead to a decrease in self-confidence and reduce aesthetic appearance. Acne patients in Indonesia in adolescents aged 15-18 years ranged from 80-85%, in women aged >25 years it was around 12%, and at the age of 35-44 years it was around 3% (Ramdani and Sibero, 2015). Women are more prone to acne than men (Lynn et al., 2016).

The cause of acne is *Propionibacterium acnes* bacteria which can be prevented by using topical preparations containing benzoyl peroxide and salicylic acid (Oon et al., 2019), and some

people also use topical antibiotics such as erythromycin and clindamycin (Madelina and Sulistiyaningsih, 2018). However, if *Propionibacterium acnes* is resistant to these topical antibiotics, it will reduce the efficacy of treatment and allow resistant transfer to other microbes. In addition, resistance to topical antibiotics against *Propionibacterium acnes* can cause resistance to other pathogenic bacteria (Oon et al., 2019). According to Madelina and Sulistiyaningsih (2018), cases of antibiotic resistance against *Propionibacterium acnes* in Indonesia are quite high.. Based on the results of his research which refers to data at Hasan Sadikin Hospital, Bandung, Indonesia, the incidence of resistance to the use of Tetracycline antibiotics is 12.9%, Erythromycin antibiotics are 45.2%, and Clindamycin antibiotics are 61.3%. The high incidence of antibiotic resistance against *Propionibacterium acnes* infection requires the use of anti-bacterial compounds contained in a plant (Marselia et al., 2015). One that has the potential as an antibacterial is the Dadangkak plant (*Hydrolea spinosa L.*).

Based on its empirical benefits, the Dadangkak plant (*Hydrolea spinosa L.*) is often used by people in South Kalimantan as an antidiabetic (Zaini et al., 2017), antipyretic (fever reducer) (Forestryana & Yunus, 2018), as an antimalaria, cough medicine for bleeding, wound medicine. , ulcer medicine, mosquito repellent, antihypertensive, and stroke (Hardarani, 2011) (Darsono & Kuntorini, 2012).

Research on the Dadangkak plant (*Hydrolea spinosa L.*) has been carried out on antibacterial activity on the leaves and stems and on the roots, in vitro studies on antidiabetic activity have been carried out. Based on research on antibacterial activity conducted by Darsono and Fajriannor (2020), the leaves of the Dadangkak contain flavonoids, triterpenoids, saponins and tannins, and have antibacterial activity against the bacteria *Bacillus subtilis*, *Staphylococcus aureus*, and *Eschericia coli*, while according to Andryanie (2020), the part Dadangkak stems have antibacterial activity against *Staphylococcus aureus* bacteria and contain alkaloids, flavonoids, triterpenoids, saponins, and tannins.. According to research conducted by Zaini et al. (2017), ethyl acetate extract of Dadangkak root has antidiabetic activity and contains tannins and saponins. Based on the compounds contained in the roots, leaves, and stems of Dadangkak, it is possible that the roots of Dadangkak also have the same secondary metabolite compounds as the secondary metabolites contained in the stems and roots, namely flavonoids, triterpenoids, saponins, tannins and alkaloids. These compounds have antibacterial activity.

Propionibacterium acnes is a gram-positive bacterium and is the main cause of acne (Zahrah et al, 2019). These bacteria are classified as gram-positive and anaerobic bacteria. If the skin pores are blocked or can't breathe, *Propionibacterium acnes* bacteria can multiply very quickly (Mumpuni and Wulandari, 2010), in addition, *Propionibacterium acnes* bacteria are able to release lipase which results in the breakdown of free fatty acids from skin lipids, the breakdown of free fatty acids cause tissue inflammation and stimulate acne (Zahrah et al., 2019).

Based on the above background, to prove that the roots of the Dadangkak root can be used as an antibacterial, the researchers tested the antibacterial activity of the extract of Dadangkak root (*Hydrolea spinosa L.*) against the acne-causing bacteria, namely *Propionibacterium acnes*.

2. Materials and Methods

Materials

Researchers used several materials as follows: aquadest, extract of Dadangkak root (*Hydrolea spinosa L.*) as research samples, *Propionibacterium acnes* bacteria as test bacteria obtained from the Microbiology Laboratory of Lambung Mangkurat University, concentrated

sulfuric acid (H_2SO_4P), HCL, $FeCl_3$ 1%, Nutrient Agar (NA), Nutrient Broth (NB), Mueller Hinton Agar (MHA), 70% ethanol, 96% ethanol, magnesium powder, Dragendorff's reagent, chloroform, DMSO (Dimethyl Sulfoxide), and Clindamycin.

Methods

The research on the antibacterial activity test of the extract of Dadangkak root (*Hydrolea spinosa L.*) against *Propionibacterium acnes* was categorized as a *true experimental study* with the research design used, namely *post test only control group* design. Researchers collect data by observation and use qualitative and quantitative data in this study. The researcher uses the *One Way Anova* data analysis method if the research data obtained are normally distributed and the data variants are homogeneous, if one or both of them do not meet the requirements, the researcher uses non-parametric data analysis by employing the *Kruskall-Wallis* test followed by the *Post Hoc Mann-Whitney* test (Desmara et al., 2017) using SPSS (Statistical Package for The Social Sciences) software.

- The Phytochemical Test of Dadangkak Root Extract (*Hydrolea spinosa L.*)

  Flavonoid Test

  2 ml of thick extract was put into a test tube and added 2 ml of ethanol. After that add magnesium powder and concentrated HCl. If the solution changes color to orange or yellow, the extract contains flavonoid compounds (Noval et al., 2019).

  Alkaloid Test

  Researchers put in enough extract, as well as a few drops of Dragendorff's reagent, if a reddish brown precipitate is formed, the extract is declared to contain alkaloid compounds (Heliawati, 2018).

  Saponin Test

  Researchers put enough extract into a test tube, 5 ml of distilled water and then shake the solution vigorously. If a stable foam is formed for 3 minutes with a height of 1 cm, the extract shows the presence of saponin compounds (Heliawati, 2018) (Forestryana & Arnida, 2020).

  Tannin Test

  Researchers put enough extract into a test tube and then add a 1% FeCl3 solution, if a dark green or blue-black color is formed, the solution is positive for tannins (Forestryana & Arnida, 2020).

  Triterpenoid Test

  2 ml extract was added with 1 ml Liebermann-Burchard reagent. If a blackish green or dark green color is formed, the sample indicates the presence of triterpenoid compounds (Ningsih et al., 2016).

- The Screening Test for Antibacterial Activity of Dadangkak Root Extract (*Hydrolea spinosa L.*)

  Antibacterial activity testing was carried out using disc paper. Paper discs were soaked with extract of Dadangkak root which had been diluted with DMSO, and the control group was clindamycin and DMSO. Mueller Hinton Agar (MHA) liquid medium was poured into a petri dish which had been sterilized aseptically as much as 20 ml and allowed to solidify. A 20µl suspension of *Propionibacterium acnes* was flattened with *L spread* on MHA solid media, then disc paper soaked with extract was placed on the surface of MHA solid media..

Each paper disc is equally spaced and aseptically. The MHA media that had been placed on the paper disk was incubated in an incubator for 24 hours at 37˚C. According to Arisanty & Dewi (2018), the presence of antibacterial activity is indicated by the formation of a clear zone in the form of a circle around the paper disc and its diameter is measured with a caliper after being incubated for 24 hours.

- The Minimum Inhibitory Concentration (MIC) Test of Dadangkak Root Extract (*Hydrolea spinosa L.*)

  Researchers used the liquid dilution method in the MIC test of the extract of Dadangkak root (*Hydrolea spinosa L.*) against the bacteria *Propionibacterium acnes*. The method of preparation of the sample solution (test group) is that each tube used is filled with a solution of 0.4 ml of Nutrient Broth (NB), 0.1 ml of a bacterial suspension solution, and a 0.5 ml test solution. As well as a negative control solution (DMSO), a positive control solution (Clindamycin), and a concentration solution of extracts of Dadangkak root (*Hydrolea spinosa L.*) 20%, 40%, 60%, 80%, and 100%. The researchers incubated the negative control solution, positive control solution, and extract solution in an incubator for 24 hours at 37˚C while observing the turbidity of each solution after incubation. According to Sariadji et al. (2019), the MIC value can be determined from the lowest concentration of the extract indicating the presence of clarity.

- The Minimum Bactericidal Concentration (MBC) Test of Dadangkak Root Extract (*Hydrolea spinosa L.*)

  The Minimum Bactericidal Concentration (MBC) can be determined after the MIC test is carried out, because the determination of the MIC requires a MIC solution, which is the lowest concentration solution of the extract that is poured and spread evenly onto solid Mueller Hinton Agar (MHA) media. Each solution used for MIC testing was taken as much as 1 ml and spread using an *L spreader* on MHA solid media. MHA solid media was incubated in an incubator for 24 hours at 37˚C. According to Zahrah et al. (2019), the MBC value can be determined from the lowest concentration of the extract which does not show any bacterial growth on MHA solid media.

3. Results and Discussion

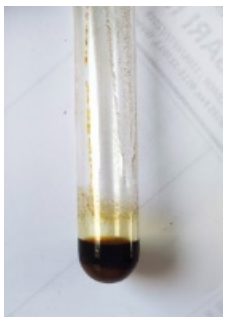
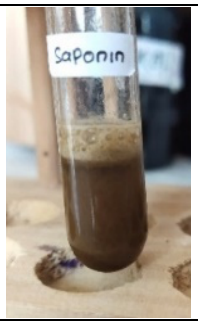
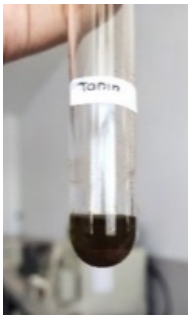
Results

- The Phytochemical Test of Dadangkak Root Extract (*Hydrolea spinosa L.*)

  Based on the results of phytochemical tests, the extract of Dadangkak root (Hydrolea spinosa L.) contains flavonoid compounds, alkaloids, tannins, and saponins. The results of the phytochemical test of the extract of Dadangkak root (Hydrolea spinosa L.) can be seen in table 1.

Table 1. The Phytochemical Test Results Dadangkak Root Extract (Source: Primary Data, 2021)

Phytochemical Name	Result	Description.	Figure

Alkaloid	Reddish brown	+	
Flavonoid	Yellow	+	
Saponin	Stable foam	+	
Tanin	Dark green	+	

Triterpenoid	Golden yellow	-	

- The Screening Test for Antibacterial Activity of Dadangkak Root Extract (*Hydrolea spinosa L.*)

The results of the screening test for the antibacterial activity of the Dadangkak root extract using the disc diffusion method showed that the Dadangkak root extract possessed antibacterial activity against the bacteria *Propionibacterium acnes*. The test results can be seen in table 2 and figure 1.

Table 1. Screening Test for Antibacterial Activity of Dadangkak Root Extract Results (Source : Primary Data, 2021)

Treatment Group	**Average diameter (mm)**	**Growth Inhibitory Response**
Dadangkak root extract	8,29 mm	Medium
Positive control (Klindamisin)	12,83 mm	Strong
Negative control (DMSO)	0 mm	Weak
Description : (Buldani dkk., 2017) Weak : <5 mm Medium : 5-10 mm Strong : 10-20 mm Very strong : ≥ 20 mm		

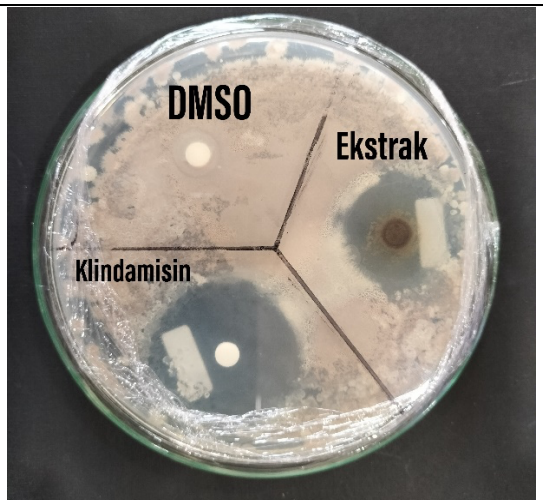


Figure 1. Minimum Inhibitory Concentration Test (MIC) of Dadangkak Root Extract (*Hydrolea spinosa L.*)

- The Minimum Inhibitory Concentration Test (MIC) of Dadangkak Root Extract (*Hydrolea spinosa L.*)

The results of the MIC test of Dadangkak root extract using the liquid dilution method showed clarity after being incubated for 24 hours using concentration 60%.

Table 2. The Minimum Inhibitory Concentration Test (MIC) of Dadangkak Root Extract Results (Source : Primary Data, 2021)

Treatment Group	Replication			*p value*
	I	II	III	
20% of concetration (0,02 g/ml)	-	-	-	-
40% of concetration (0,04 g/ml)	-	-	-	-
60% of concetration (0,06 g/ml) (MIC)	+	+	+	0,018[a] 0,025[b]
80% of concetration (0,08 g/ml)	+	+	+	0,018[a]
100% of concetration (1 g/ml)	+	+	+	0,018[a] 0,025[c]
Positive control (Clindamycin)	+	+	+	-
Negative control (DMSO)	-	-	-	-

Description :
(-) The turbidity is being in solution
(+) The clarity is being in solution
([a]) *p value* in *Kruskall-Wallis* test
([b])*p value* of MIC data analysis at 60% of concetration and 80% of concetration with *Mann-Whitney* test
([c]) *p value* of MIC data analysis at 80% of concetration and 100% of concetration with *Mann-Whitney* test

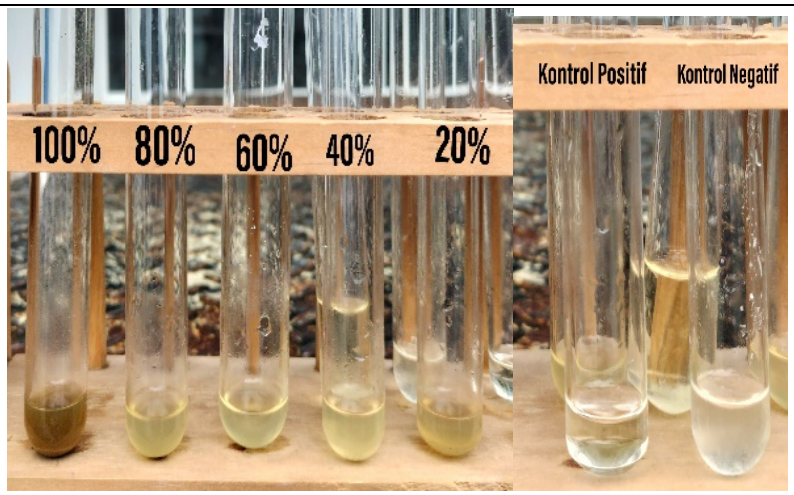


Figure 2. The Minimum Inhibitory Concentration Test (MIC) of Dadangkak Root Extract Results (Source : Primary Data, 2021)

- The Minimum Bactericidal Concentration (MBC) Test of Dadangkak Root Extract (*Hydrolea spinosa L.*)

The results of the KBM test of Dadangkak root extract using the solid dilution method showed that all concentrations and negative controls (except positive controls) were overgrown by bacteria after being incubated for 24 hours.

Table 3. The Minimum Bactericidal Concentration (MBC) Test of Dadangkak Root Extract (Source : Primary Data, 2021)

Treatment Group	Replication		
	I	II	III
20% of concetration (0,02 g/ml)	-	-	-
40% of concetration (0,04 g/ml)	-	-	-
60% of concetration (0,06 g/ml)	-	-	-
80% of concetration (0,08 g/ml)	-	-	-
100% of concetration (1 g/ml)	-	-	
Positive control (Clindamycin)	+	+	+
Negative control (DMSO)	-	-	-
Description : (-) The turbidity is being in MHA solid media (+) The clarity is being in MHA solid media			

Discussion

- The Phytochemical Test of Dadangkak Root Extract (*Hydrolea spinosa L.*)

Phytochemical test of extract of Dadangkak root (*Hydrolea spinosa L.*) was carried out qualitatively (using color reaction). The results obtained in the phytochemical test showed that the extract of Dadangkak root was positive for flavonoid compounds, alkaloids, tannins, and saponins. These four compounds have antibacterial activity with different mechanisms. Flavonoid compounds are able to form complex compounds with extracellular proteins, damage cell membranes, and activate enzymes (Indarto et al., 2019), alkaloid compounds are able to interfere with the peptidoglycan constituents of bacterial cells which results in the formation of cell walls in bacteria being incomplete (Putri, 2017). Tannins are able to interfere with polypeptides in the bacterial cell wall so that the formation of the cell wall becomes imperfect so that the bacteria will die (Soekamto et al., 2017) and are able to coagulate and denature proteins by binding to proteins to form H+ bonds resulting in a change in pH to acid. An acidic pH will inactivate bacterial enzymes that can disrupt the metabolism of bacterial cells and damage cells and lead to bacterial death. Tannin compounds are able to inhibit *the reverse transcriptase* and DNA *topoisomerase* enzymes which cause cells in bacteria to not be formed (Indarto et al., 2019). Saponin compounds work as antibacterial by interfering with cell permeability so that cell transport is disrupted and is capable of lysis of bacterial cells (Andalas, 2019).

- The Screening Test for Antibacterial Activity of Dadangkak Root Extract (*Hydrolea spinosa L.*)

Researchers used the disc diffusion method to examine the antibacterial activity of the extract of Dadangkak root. The antibacterial activity of the extract can be seen from the presence of an inhibitory zone formed around the disc after incubation (Rollando, 2019). Based on the results of the screening test, the extract of Dadangkak root showed a clear area around the disc. The area of the diameter zone of the extract of the dadangak root obtained was 8.29 mm. The diameter of the resulting inhibition zone has a moderate category of antibacterial activity. According to Buldani et al. (2017), the antibacterial power with an inhibition zone diameter of 5-10 mm is in the medium category. According to Rollando (2019), the effectiveness of antimicrobials is indicated by the presence of an

inhibitory zone formed around the disc after incubation, the wider the inhibition zone it produces, the more sensitive the compound is.

- The Minimum Inhibitory Concentration Test (MIC) of Dadangkak Root Extract (*Hydrolea spinosa L.*)

  The researcher carried out the test to determine the lowest concentration of extract that was able to inhibit the growth of *Propionibacterium acnes* bacteria which was characterized by minimal bacterial growth compared to the control solution (Zahrah et al., 2019). The MIC value was determined from the concentration of the extract which showed clarity in the media (Sariadji et al., 2019). Based on the MIC test that has been carried out, the dadangak root extract with a concentration of 60% shows clarity so that it can be concluded that the MIC value of the Dadangkak root extract is at a concentration of 60%. The antibacterial activity of Dadangkak root extract is the same as that of Fitriyanti et al. (2019) and Azrifitria et al. (2010). Based on the research of Fitriyanti et al. (2019) which used the same method and bacteria, but with different extracts and concentrations, the MIC value of old soursop leaf infusion (Annona muricata L.) was at a concentration of 25%, while in the study conducted by Azrifitria et al. (2010) who used the same method but different concentrations and extracts, the MIC value of the ethanolic extract of white lily (*Crinum asiaticum L.*) leaves was at a concentration of 1.25 mg/ml and the MIC value of the ethanolic extract of lily bulbs was at a concentration of 7.5 mg/ml.

  After being incubated for 24 hours, the positive control solution (Clindamycin) showed clarity because Clindamycin is a bacteriostatic and bactericidal antibiotic (inhibits and kills bacterial growth) (Mulyani et al., 2017) and Clindamycin is commonly used to treat acne (Madelina & Sulistiyaningsih, 2018) , and that's why clindamycin was used as a positive control in the MIC test, while the negative control solution (DMSO) showed turbidity after incubation because DMSO did not have antibacterial properties so it was suitable to be used as a negative control (Sundu & Handayani, 2018). The results of the MIC test carried out normality and homogeneity tests to determine whether the data obtained in this study met the requirements to be tested statistically using *One Way Anova*. The results of the normality test in this research data using *Shapiro Wilk* (Desmara et al., 2017) and the results of the normality test in this research data are zero, as well as the homogeneity. This shows that the data is not normally distributed and not homogeneous, as a result, data analysis with *One Way Anova* cannot be carried out so that data analysis in this study uses non-parametric data analysis, namely the *Kruskall-Wallis* test followed by the *Pos Hoc Mann-Whitney* test. The statistical test results obtained by the *Kruskall-Wallis* test were 0.018 (p value below 0.05). This shows that all extract concentrations have an effect on inhibiting the growth of *Propionibacterium acnes*, while the results of the *Mann-Whitney* test using 60% and 80% concentration data and 80% and 100% concentration data both show a significant value of 0.025 (p value < 0.05), this indicates that each concentration has a significant difference to inhibit *Propionibacterium acnes*.

- The Minimum Bactericidal Concentration Test (MIC) of Dadangkak Root Extract (*Hydrolea spinosa L.*)

  The MBC test of Dadangkak root extract against *Propionibacterium acnes* was carried out using the solid dilution method. This test was conducted to determine the lowest concentration of the extract which was indicated by the absence of bacterial growth on

Mueller Hinton Agar media in petri dishes that had been inoculated with the test preparation. The lowest concentration of extract capable of killing bacteria was indicated by *Propionibacterium acnes* bacteria could not grow on the media in the petri dish because the test solution succeeded in killing the bacteria (Zahrah et al., 2019). Based on the MBC test that has been carried out, all media that have been immersed in a concentrated extract solution (except positive controls) are covered with *Propionibacterium acnes* bacteria. This indicates that all the concentration materials employed were unable to kill Propionibacterium acnes. The results of the MBC test are also similar with the results of the research by Mulyani et al. (2017) which is where the extract used is the same but the concentration used is also the same as the concentration of the extract used in this MBC test. Based on the results of research conducted by Mulyani et al. (2017), the MBC value of katuk leaf extract (*Sauropus androgynus L*. (*Merr*)), all the employed concentrations, namely 20%, 40%, 60%, 80%, and 100% did not have an inhibition zone that made the MBC test discontinued.

4. Conclusion

The conclusion of this research is that the extract of Dadangkak root has potential as an antibacterial against *Propionibacterium acnes*. Dadangkak root extract has antibacterial activity which is indicated by the presence of a clear area around the disc because it contains secondary metabolites, namely flavonoids, alkaloids, tannins and saponins. The MIC value of Dadangkak root extract was at a concentration of 60% (0.06 g/ml) and there was no MBC value for the root extract because all the media used at all concentrations were overgrown with *Propionibacterium acnes* bacteria.

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Declaration of Interest Statement

The authors declare that they have no conflict of interests.

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