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Antimicrobial Activity of Eco-enzymes with Various Dilutions As A Natural Disinfectant

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Abstract: Ecoenzyme perfectly utilizes biological waste for non-chemical sanitation products through the fermentation of sugar, organic materials, and water. Ecoenzyme has been used to inhibit the growth of bacteria such as Staphylococcus aureus and Propionibacterium acnes. This study aims to determine the optimum concentration of eco enzyme as a natural antibacterial and disinfectant. This method uses gram-positive bacteria Staphylococcus aureus and gram-negative bacteria Escherichia Coli with the disc method. The dilution variations used were 0, 1:10, 1:50, and 1:100. Ecoenzyme is made from organic materials from pineapple, orange, papaya, starfruit, quinine, and mango skin waste (Bioz1), and coenzyme from pineapple, orange, banana stem, noni, quinine waste (Bioz2). The results showed that a ratio of 1:10 was the most effective dilution variation for inhibition against both types of bacteria. Furthermore, dilution at this concentration can be used as a natural disinfectant. The presence of acetic acid and enzymes (i.e. lipase and amylase) can inhibit certain strains of microorganisms, namely Escherichia coli and Enterococcus sp.

Keywords: Antibacterial; Eco-enzyme; Organic; Natural disinfectant; Variation.

Introduction

Disinfection is an effort to clean microorganisms such as bacteria, fungi, and viruses from the surface of objects using disinfectant materials (Jabłońska-Trypuć et al., 2022; Saravanan et al., 2021). Disinfectants are only used on inanimate objects; they should not be used on humans or other living things, and antiseptic substances are used for living things (Dhama et al., 2021). According to WHO, disinfectant liquid can kill bacteria and viruses within 10-60 minutes after spraying. This is because of the effect of chlorine content in bleach. The WHO disinfectant formula is then widely used by the public to clean the home environment. However, in practice, people spray disinfectant not only on rooms or objects but also on the body or limbs.

Regarding this practice, which is increasingly widespread, WHO emphasized that spraying disinfectant liquid all over the body will not kill the coronavirus. Because the virus has entered the body, while the disinfectant liquid only reaches outside the body, disinfectants kill viruses on the surface of objects. Spraying these chemicals can be dangerous if they come into contact with clothing or mucous membranes such as the eyes or mouth. Chlorine-containing disinfectant fluids to the body can have adverse effects on health. Alcohol and chlorine can be helpful as disinfectants on surfaces, but only used according to the instructions.

Based on the description above, a natural disinfectant made from organic materials is sought that is not harmful if it comes into direct contact with the skin or clothing. It is even safe if it enters the mouth to be gargled in large or small amounts. It is also environmentally friendly and can function as an antibacterial or antifungal(Hoffman et al., 2008; Mosaddad et al., 2023). One of the natural products that can be used as a natural disinfectant is eco enzyme.

Eco enzyme is a multipurpose liquid fermented from organic materials in the form of fruit peel waste or vegetable waste, sugar and water (Benny et al.,

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2023)(Barman et al., 2022). The raw materials used in making ecoenzymes significantly affect the success of the fermentation process in eco-enzymes. The resulting eco-enzyme liquid is usually brown or based on the organic materials used (Benny et al., 2023). The characteristic results of eco-enzymes that have been fermented for at least 100 days have met the requirements of good eco-enzymes, namely pH <4 and a distinctive fresh sour aroma like other fermentation results (Srihardyastutie & Rosmawati, 2024). The phenol content contained in the eco-enzyme liquid effectively reduces bacterial growth to be used as a disinfectant. In the 1:10 sample dilution in the antibacterial test, it was seen that only a few bacteria or microorganisms grew. The results of the disinfectant quality characteristics, namely pH, and the stability of the complex water emulsion for each sample have met the requirements based on S.N.I. 06 - 1842 Year 1995, namely pH 6 - 11 and stable emulsion stability in hard water. At sample dilution of 1:10, 1:20, 1:30, 1:40 and 1:50, the pH value obtained is 6-7, where the value meets the quality requirements of disinfectants. Under challenging conditions of water stability testing, all disinfectant product samples have stable emulsion stability in hard water because there is no formation or absence of sediment or layers in the disinfectant product samples, and they meet the quality requirements of disinfectants (Watkinson, 2008).

The ability of the eco enzyme to inhibit bacterial and fungal activity is related to its ability as a (natural) disinfectant, however, Ecoenzyme also has a nutritional content in the form of 6.960% crude fibre, 7.538% crude protein, and 0.321% crude fat (R. B. Ginting et al., 2023), as a multifunctional liquid; other uses of Ecoenzyme are**:** in the cultivation of perennial plants (Warsito et al., 2023), increasing the fertility of shallot plants with a ratio ofEcoenzyme: water is 1:100 (Lubis, et al., 2022), and soybean plants(Lubis, et al., 2022), 75% topsoil + 25% compost soil treatment with the provision of ecoenzymes can respond to growth such as plant height and several red onion leaves(Luta et al., 2022), manufacture Ecoenzyme Using household organic waste to support sustainable agriculture in Suka Damai Village, Langkat (Wasito, 2023), and functions as liquid organic fertilizer (Hakim et al., 2023).

Types of waste that can be used in manufacturing, such as ecoenzyme among others, are derived from plant waste such as citrus fruit, pineapple, papaya, and other organic waste (Permatananda et al., 2023; Siregar et al., 2024). Citrus waste can be used because it contains bioactive compounds such as flavonoids that play a role in antimicrobial activity. The mechanism of flavonoid inhibition as an antibacterial agent involves inhibiting nucleic acid synthesis, disrupting the function of the cytoplasmic membrane, and affecting the formation of biofilms, porins, permeability, and interactions of bacterial enzyme work (Górniak et al., 2019; Shamsudin et al., 2022). In synthesis, coenzymes can be used as antibacterials, antiseptics, and agents for reducing worm egg contamination in fresh fruits and vegetables. Ecoenzymes contain lipase, protease, and amylase enzymes, which can cause damage to the structure of microbial cells. They can also be used as an environmentally friendly fruit and vegetable washing liquid. Waste enzyme production through fermentation is an efficient path to convert organic waste into value-added products in environmental matrices, including organic degradation, composting, wastewater treatment, and disinfection processes (Pasalari et al., 2024).

Ecoenzymehas been found to have significant antibacterial activity against several types of bacteria. The ability of coenzymes to inhibit bacteria makes it possible for them to function as a natural disinfectant and hand sanitizer (Donaghy et al., 2019). Ecoenzymes have been studied for their potential as disinfectants against pathogenic microbes, including Staphylococcus aureus and Candida albicans. B.S. Aureus bacteria is known to cause skin infections and several other diseases. The study results showed that this eco enzyme has the potential as a natural antibacterial that can be used in the treatment and preservation of food and in other applications that require microbial control. Organic waste that can be used to make eco enzymes as antibacterials includes horticultural plant waste in the form of fruit and vegetable peels. Other studies support that eco enzymes made from a mixture of fruit and vegetable peel waste with water and molasses can be used as effective antibacterials against Staphylococcus aureus and Staphylococcus epidermidis, the cause of skin infections.

In several studies, coenzymes have been used to inhibit the growth of bacteria such as Staphylococcus aureus and Propionibacterium acnes.(Ramadani et al., 2022). The results of the study showed that the eco enzyme of pineapple (Ananas compass L) and orange (Citrus X Sinensis L) waste could inhibit bacterial growth at different concentrations, with the highest concentration (60%) showing the most effective results in inhibiting the growth of Staphylococcus aureus bacteria by 13.1 mm, with the formation of a clear zone around the disc.(Mubarokah & Halimatussa'diah, 2023).Ecoenzyme has also been found to have antibacterial potential because it has a low pH, which can damage the flexibility of bacterial cell walls and inhibit their growth. In addition, the eco enzyme can also be used as an antiparasitic agent, such as in inhibiting the growth of Ascaris lumbricoides, with high concentrations of acetic acid effective in damaging the cell walls of parasite eggs. Eco Enzyme, from pineapple

(Ananas comosus), banana (Musa paradisiaca) and papaya (Carica papaya) waste at concentrations of 1:100, 1:200, or 1:300, effectively inhibits Staphylococcus aureus. With a dilution variation of 1:100 showing the most significant inhibition power(N. Ginting & Prayitno, 2022).

However, research on Ecoenzymes that mentions what type of fruit waste is used and at what concentration variations are effective for natural antibacterial or disinfectants still needs to be completed. So, this study uses waste determined by type and combined with banana stems and other samples combined with the flesh of the noni plant. The resulting Ecoenzymes will be diluted with different concentration variations, and their activity will be tested against bacteria *S.aureus and E.coli*to to find out at what concentration coenzyme is most effective as a natural antibacterial and disinfectant.

Methods

This research was conducted in 2 stages; stage 1 was the manufacture of coenzymes conducted at the Agrotechnology Laboratory, Universitas Pembangunan Panca Budi, Medan. While the second stage of research, namely the antimicrobial activity test of eco enzymes, was conducted in vitro at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara.

Materials and tools

The materials used to manufacture eco enzymes are organic materials from agricultural products and waste, carbohydrates, and mineral water—isolates of S. aureus bacteria and C. albicans lines from the Universitas Sumatera Utara Microbiology Laboratory. The tools used are knives, scales, measuring cups, nutrient agar, M.H.A., M.R.S.A., M.R.S.B., Petri dishes, O.S.E. needles, autoclaves, pH meters, and disc paper.

Ecoenzyme Production

The principle of making Eco enzyme is 3:1:10, namely 3 kg of organic materials from agricultural products and waste, 1 kg of carbohydrates (molasses), and 10 L of mineral water. Ecoenzyme is made from organic waste materials. Pineapple, orange, papaya, starfruit, quinine, mango skin (sample code Bioz1); and eco enzymes from pineapple, orange, banana stem, noni, quinine waste (sample code Bioz2). The organic materials are washed or cleaned, cut into small pieces, and then weighed with a total weight of 3 kg of organic materials. Prepare a plastic container filled with 10 L of water, and put molasses into the container filled with water, stir until the molasses dissolve in the water. After all the ingredients are homogeneous, the container is tightly closed for the fermentation process for 100 days.

Fermentation takes place at room temperature and anaerobic conditions.

After the fermentation time is complete, the container is opened, and the coenzyme solution is filtered to separate the coenzyme filtrate and dregs (residue). The filtered coenzyme is placed in a plastic container that is ensured to be dry and clean.

Ecoenzyme dilution

The pure eco enzyme (Bioz-0) was diluted with several variations of dilution ratios: 1:10 by dissolving 10 mL of the eco enzyme into 100 mL of distilled water (Bioz1-1), 1:50 by dissolving 2 mL of the eco enzyme into 100 mL of distilled water (Bioz1-2), and 1:100 by dissolving 1 mL of the eco enzyme into 100 mL of distilled water (Bioz1-3).

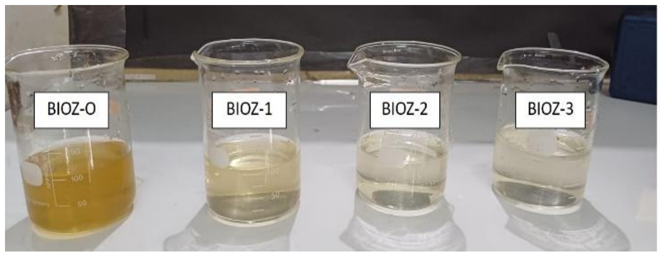

Figure 1. Ecoenzyme dilution variation

Preparation of standard solutions

Preparing McFarland standard solutions standardizes the estimated number of bacteria in a suspension fluid. This McFarland turbidity standard is intended to replace counting bacteria one by one and estimate the cell density used in antimicrobial testing procedures. The number of McFarland standards 0.5 equals 1.5 x 108 bacterial cells.

The first step is to prepare a test tube and pour distilled water into each test tube labelled with bacteria. Using an ose, put each bacteria into each test tube labelled according to the type of bacteria, homogenizing it. Then, observe the turbidity of the dilution solution and equalize it with the existing 0.5 McFarland standard until the turbidity is the same.

Testing the Effectiveness of Lactic Acid Bacteria Against Pathogenic Bacteria

They make M.H.A. (Mueller-Hinton Agar) media by weighing 7.2 grams and adding 250 mL of distilled water, then homogenizing and heating until clear. M.H.A. media is poured into 12 Petri dishes to be used, which is left to solidify. Antibiotic discs (positive control) chloramphenicol and blank discs are prepared for antibacterial activity testing. Each colony of test bacteria (pathogenic bacteria) that has been diluted.

According to McFarland standards, 0.1 mL (10 µL) was taken using a micropipette and spread using an L rod on M.H.A. (Muller et al.) media. In 12 M.H.A. (Mueller-Hinton Agar) media, holes (wells) were made using a sterile perforator; each media was made with two holes. Chloramphenicol antibiotic discs were taken using sterile tweezers and placed on the middle surface of the M.H.A. (Mueller-Hinton Agar) media as a positive control. All ready samples were then incubated with the dish not inverted at 37 ° C for 24 hours. After the incubation period, an inhibition zone will appear. The diameter of the inhibition zone that formed was measured using a vernier calliper at 24 hours and 48 hours. The inhibition zone was measured twice. The data listed in Table 1 are the average diameter of the inhibition zone as a result of the gram-positive and gram-negative antibacterial activity test.

Results and Discussion

Results of the Ecoenzyme antibacterial activity test against bacteria *Escherichia coi*l are shown in Table 1.

Table 1. Antibacterial activity test results from enzyme

Sample code	Inhibition zone diameter (mm)	
	Escherichia coli	*Staphylococcus aureus*
BIOZ1		
BIOZ1-0	0	0
BIOZ1-1	7.9	6.3
BIOZ1-2	0	0
BIOZ1-3	0	0
BIOZ2		
BIOZ2-0	0	0
BIOZ2-1	7.2	6.5
BIOZ2-2	0	0
BIOZ2-3	0	0
chloramphenicol (control)	27	28

Table 1 shows that Ecoenzyme from Bioz-1 (pineapple peel, orange, papaya, starfruit, quinine, and mango waste) and Bioz-2 (pineapple, orange, banana stem, noni, and quinine waste) showed inhibition against E. coli bacteria with an average diameter of 7.9 mm (Bioz1-1) and 7.2 mm (Bioz2-1), and showed inhibition against S. aureus bacteria measuring 6.3 mm (Bioz1-1) and 6.5 mm (Bioz2-1) in Figure 3, it is in the moderate category. The inhibition of Bioz1-1 bacteria is slightly higher than Bioz1-2 when viewed from the type of organic material from Ecoenzyme, where the Bioz1-1 sample is more than Bioz1-2. This can be seen by forming a clear zone around the well. The inhibition zone in Bioz1 was larger than Bioz2 in both types of bacteria and at the same concentration; this was likely due to the presence of organic matter from papaya contained in Bioz1. A study reported that papaya contains phytochemical compounds such as phytosterols, tocopherols, flavonoids, alkaloids, and carotenoids (Misnan et al., 2024; Thanigaimalai et al., 2025).

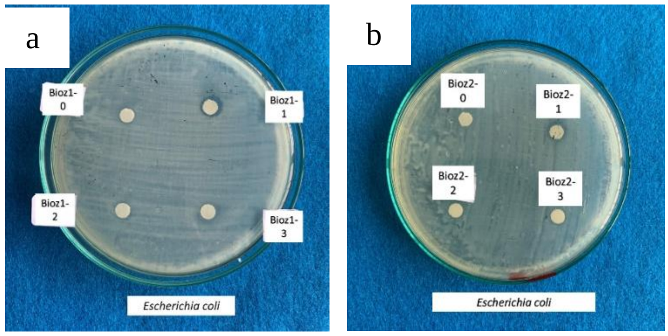

Figure 2. Antibacterial activity test Ecoenzyme against E.coli bacteria

On Ecoenzyme pure (1:0) has no inhibitory power. Likewise, other dilutions have no inhibitory power against E. coli bacteria. Eco enzyme with a solution dilution ratio of 1:10 (Bioz-1 and Bioz-2) is the best and most practical (optimal) dilution, with the presence of an inhibition zone even though it is in the moderate category because it is in the 5-10 mm zone (N. Ginting & Mirwandhono, 2021; Varshini & Gayathri, 2023; Vimalanathan et al., 2025). This is likely due to the material Ecoenzyme contains fruit skin and not vegetables. This is in line with previous research, which stated that Ecoenzyme from orange peel, pineapple, and papaya contain phenol contained in eco enzyme liquid and are effective in suppressing bacterial growth so that they can be used as natural disinfectants. In the 1:10 sample dilution in the antibacterial test, only a few bacteria or microorganisms grew (Varshini & Gayathri, 2023; Vimalanathan et al., 2025), as seen in Figure 2 and Figure 3. Pure eco-enzyme has no inhibitory power against E. coli and S. aureus bacteria; it is possible that the good bacteria that produce organic acids are not yet active because they have not been mixed with distilled water. There is a difference in the 1:10 dilution because the good bacteria are less active in other dilutions of 1:50 and 1:100 because of the high percentage of distilled water eco enzyme. Alcohols, phenolics, organic acids, and hydrogen peroxide (H2O2) are among the compounds found in the coenzyme. Because of these active ingredients, Ecoenzyme has antibacterial properties and can be a natural disinfectant that is safe for human contact and the environment. It is made from organic materials easily found in the home environment. Therefore, Eco-enzyme is highly recommended for use at home.

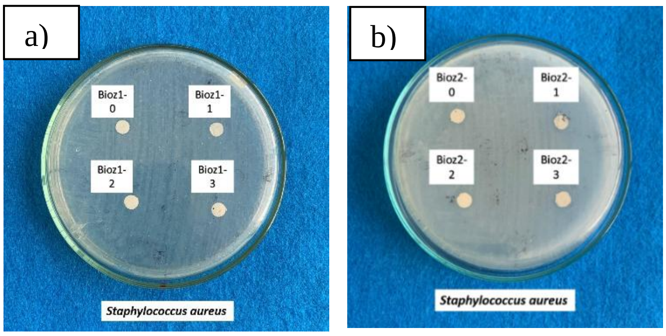

Figure 3. Antibacterial activity test Ecoenzyme against staphylococcus aureus bacteria

This is slightly different from the results of previous studies, which reported that eco-enzymes derived from pineapple skin are known to contain tannins and saponins and have antibacterial activity, as evidenced by their ability to inhibit the growth of P. aureus and S. aureus acne bacteria at minimum inhibitory concentrations (M.I.C.) of 50% and 100% (v/v), respectively. However, no effective concentration was found in P. Acnes in our study, we used eco-enzymes made from five different types of organic materials, while the organic materials (waste) used in this study consisted of only one type. The more diverse the types of waste used in the production of eco-enzymes, the richer the types of organic and phytochemical content produced so that they can inhibit the growth of pathogenic bacteria. As stated by Rijal (2022), eco-enzymes from eucalyptus leaves have an inhibitory cone of 14.42 mm for E. coli and 12.58 mm for S. Aureus (Ammar et al., 2024; Pandey & Chandra, 2023). The antimicrobial activity of eco-enzymes has been demonstrated.

In this study, the main components used in the manufacture of eco-enzymes include various fruits and vegetables, including papaya, soursop, banana, orange, pineapple, and other ingredients containing bioactive substances that affect microbial activity, such as flavonoids, tannins, saponins, pH, and lactic acid. These substances work by inhibiting nucleic acid synthesis, cell membrane function, bacterial energy metabolism, and cross-bacterial cell membranes caused by pH gradients, which disrupt bacterial cellular metabolic activity. Therefore, eco-enzymes can inhibit bacterial growth by forming a clear zone around the disc. Therefore, Eco-enzymes can be natural disinfectants in our environment [20].

The use of fruit and vegetable waste to make eco-enzymes is generally used in the agricultural sector as a liquid fertilizer and water purifier (wastewater treatment) as a biocatalyst made from orange waste, which can potentially reduce oil and fat in domestic wastewater.(Wikaningrum & Pratamadina, 2022). The use of eco-enzymes as a disinfectant is found in several studies. Disinfectant is a chemical commonly used to kill microorganisms and can eliminate 60%-90% of microorganisms (bactericide). It is widely used for sanitation in households, laboratories and hospitals and is intended for inanimate objects, such as floors, clothes and plates.(Agustina et al., 2021). Chemical disinfectants in their use can leave residues and potentially harm health. In contrast, plant-based disinfectants from natural ingredients, such as eco-enzymes, do not leave residues because they evaporate quickly.(Nofita et al., 2023; Râpă et al., 2024). According to(Safitri et al., 2021), the disinfectant solution can be made with a ratio of 50%: 50% by mixing 250 ml of eco-enzyme and 250 ml of clean water that can be used directly. The benefits of eco-enzyme as an antibacterial are also explained in the study(Ramadani et al., 2022), where the eco-enzyme from pineapple skin contains tannins and saponins also have antibacterial activity, which can inhibit the growth of acne bacteria S.aureus and P.acnes. Research results(Rahayu & Situmeang, 2021)produce a combination of eco-enzymes from organic waste composition of rambutan fruit skin, corn cobs, chayote skin with the addition of 10% frangipani sandalwood extract can inhibit the growth of Staphylococcus aureus bacteria with extreme inhibitory power (31.85-34.41 mm) to overcome the high need for disinfectants during Covid-19. In addition to sprayed disinfectants, several studies have also shown that using eco-enzymes for liquid soap supports the habit of washing hands with soap. Utilization of eco-enzymes as liquid soap(Nurulita et al., 2022)and antiseptic soap in the form of bar soap with eco-enzyme as a supporting ingredient to improve the quality and value of the product (Sukohar et al., 2024) will reduce soil pollution, help reduce the use of chemicals that are dangerous and can damage the environment, and the application of both eco-enzyme soaps is carried out directly to the community in the form of training.

In some recent articles, eco-enzyme development only discusses the use and manufacture in the form of training. However, this study has measured how effective eco-enzyme is as an environmentally friendly natural disinfectant in everyday life to reduce the number of E. coli bacteria. The results of this study are supported by previous research by(Mahdia et al., 2022)) that eco-enzymes from orange waste used as cage cleaning fluid have the ability to inhibit the growth of E. coli and Staphylococcus aureus bacteria with a reduction in the number of bacteria five times greater than using detergent.

This study's results align with the research(Nurhayati et al., 2023). The research results show that the ecoenzyme sample of pineapple skin waste (Ananas comasus) can inhibit the test bacteria. This is evidenced by the formation of a bacteria-free area (clear zone) around the disc. The results of the study showed that the inhibition zone in S. aureus bacteria with a concentration of 100% was 12.33 ± 1.7 mm with a strong category, a concentration of 75% was 10.17 mm with a strong category and at a concentration of 50% was 9.67 mm with a moderate category. Compared to this study, there is a difference in the diameter of the inhibition zone. In previous studies, the results of the average diameter of the inhibition zone at a concentration of 50% were 9.67 mm with a moderate category. In contrast, this study obtained an average diameter of the inhibition zone of 3.75 mm with a weak category. This difference can be caused by several factors, such as immersing the disc in the coenzyme and the uneven scratching factor of the bacterial suspension, which can cause no antibacterial effect around the disc. This study also aligns with the study(Amanda, 2023) about the effect of variations in eco enzyme components on the antibacterial activity of Staphylococcus aureus and Escherichia coli.

The formation of inhibition zones at each concentration results from active compounds owned by coenzymes, such as saponins and tannins. These contents have activities that can be used as antibacterials. The function of saponins as antibacterials is to work by damaging the porin in the outer membrane of the bacterial cell wall by forming a solid polymer bond. Bacterial growth is inhibited or dies due to damage to the porin as a bridge for the entry and exit of compounds, causing a nutritional crisis in bacterial cells. Tannins/phenolics as antibacterials thwart the formation of bacterial cells by blocking the work of D.N.A. topoisomerase and reverse transcriptase enzymes.

Conclusion

Eco enzyme with a concentration variation of 1:10 effectively inhibits the growth of Escherichia coli and Staphylococcus aureus bacteria using the disc method. It can be used as a natural disinfectant that is environmentally friendly, safe on the skin, and uses organic ingredients easily obtained in the household environment.

This research suggests testing the antifungal activity on the same type of fruit waste and concentration to obtain the standard concentration of eco enzyme as a natural disinfectant. Materials from fruit waste and other plant parts, such as vegetables, were used to test the concentration variation on antibacterial activity.

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Author Contributions
Preparation, proposals, methodology, article N.L. ; data collection Data analysis and preparation of articles R.D; Correction of data results S.W.

Conflicts of Interest
The authors declare no conflict of interest

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