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Isolation and Antimicrobial Activity Test of n-Hexane of Pineapple Core *Ananas comosus* L. Merr

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Abstract: Pineapple (*Ananas comosus* L. Merr) contains various bioactive compounds, including the enzyme bromelain. Pineapple pith was chosen as a sample because it is still underutilized. This study aims to isolate secondary metabolite compounds from n-hexane extract of pineapple bark and test its antimicrobial activity. Maceration was carried out using n-hexane solvent, then the extract was separated by gravity column chromatography, resulting in four fractions. Separation of pure compounds using thin layer chromatography (KLT) with n-hexane:ethyl acetate (4:1) eluent. The second fraction produced a white crystalline compound (Ac-EH) with a melting point of 128-130°C. Characterization with UV-Vis showed absorption at λmax 268 nm and 206 nm, while FTIR identified the functional groups C-O (1047 cm^{-1}), CH_3 (1379 cm^{-1}), CH_2 (1465 cm^{-1}), C-H aliphatic (2939 cm^{-1}), and OH (3309 cm^{-1}). Phytochemical tests showed that Ac-EH belongs to the terpenoid group. Antimicrobial activity tests against *Staphylococcus aureus, Staphylococcus epidermidis*, and *Candida albicans* were carried out by diffusion method at concentrations of 20%, 30%, and 40%. The results showed that the n-hexane and Ac-EH extracts were active in inhibiting bacterial growth but not against fungi. The n-hexane extract inhibited *S. aureus* by 9.1 mm, 9.5 mm, and 9.8 mm and *S. epidermidis* by 8.1 mm, 8.4 mm, and 8.7 mm. The Ac-EH compound inhibited *S. aureus* by 8.5 mm, 8.9 mm, and 9.4 mm and *S. epidermidis* by 8.3 mm, 8.7 mm, and 9.1 mm.

Keywords: Antimicrobial; Diffusion; Isolation, Pineapple core extract; *S. aureus*; *S. epidermidis*

Introduction

Pineapple (*Ananas comosus* L. Merr) is a tropical plant that is widely cultivated in Indonesia and has high economic value. Apart from the consumed pulp, other parts of the plant, such as the peel and pith, are often underutilized waste. Pineapple pith is known to contain various bioactive compounds, including bromelain, flavonoids, tannins, and saponins, which have potential as pharmacological agents, one of which is antimicrobial (Hani & Novita, 2022). However, the utilization of pineapple core in the health sector, especially as a source of active compounds with antimicrobial activity, is still very limited. Therefore, further research is needed to isolate and characterize the active compounds in pineapple stem extract and test their potential as antimicrobial agents.

In recent years, microbial resistance to antibiotics has been increasing, posing a serious threat to global health. Pathogenic bacteria such as *Staphylococcus aureus* and *Streptococcus epidermidis* have shown resistance to various conventional antibiotics, while *Candida albicans*, as an opportunistic pathogenic fungus, has also become a major cause of nosocomial infections in individuals with weak immune systems (Rahmat et al., 2016). Therefore, the exploration of natural sources with antimicrobial potential is very important to find new

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treatment alternatives that are more effective and environmentally friendly.

Several previous studies have shown that pineapple stem extract has antibacterial activity, but studies on the isolation and characterization of specific bioactive compounds from pineapple stem n-hexane extract are still limited (Minarni & Rosmalia, 2022). In addition, most of the previous studies mostly tested antibacterial activity against Escherichia coli and Streptococcus sanguinis, while research on the effectiveness of pineapple stem extract against *S. aureus*, *S. epidermidis*, and C. albicans is still very limited. In fact, these three microorganisms are the main cause of various skin infections, nosocomial infections, and opportunistic diseases in humans. Therefore, this study aims to fill the gap by isolating active compounds from the n-hexane extract of pineapple core, characterizing them, and testing their antimicrobial activity against pathogenic bacteria and fungi that cause infections.

Isolation of active compounds from pineapple stem extract aims to obtain specific bioactive components that are purer so that they can be more effective in inhibiting the growth of pathogenic microorganisms (Juariah & Wati, 2020). In this study, extraction will be carried out using n-hexane to obtain non-polar compounds that have potential as antimicrobials. Furthermore, compound isolation is carried out through column chromatography, and the results will be characterized using UV-Vis and FTIR spectrophotometers to determine the chemical structure. The identification of functional groups in these compounds will provide insight into their potential mechanism of action in inhibiting microorganisms.

Antimicrobial activity testing in this study will focus on *Staphylococcus aureus*, *Streptococcus epidermidis*, and *Candida albicans* by disc diffusion method at various concentrations. *Staphylococcus aureus* and *Streptococcus epidermidis* are pathogenic bacteria that often cause skin infections, wound infections, and nosocomial infections that are resistant to antibiotics, while *Candida albicans* is a pathogenic fungus that can cause systemic and superficial infections. By conducting antimicrobial activity tests against these three microorganisms, it is hoped that this study can provide more comprehensive information about the effectiveness of pineapple stem extract and isolated compounds in inhibiting the growth of pathogenic bacteria and fungi.

The results of this study are expected to contribute to the development of natural antimicrobial agents that can be used as an alternative in handling microbial infections. In addition, the utilization of pineapple core waste also has the potential to increase the added value of agricultural industry by-products and support the principle of sustainability in the utilization of natural resources. Thus, this research not only has scientific value in the fields of pharmacy and microbiology, but also has an impact on environmental and economic aspects.

Method

This research uses experimental laboratory methods. using several test parameters that are carried out in detail.

Making simplisia

Sample Pineapple hump was obtained from a pineapple seller in the rimbo panjang area of Kampar Riau, the pineapple hump had been removed. The pulp was dried in a cupboard dryer at 45°C for 48 hours. The dried pineapple pith was made into powder (Naritasari et al., 2018).

Sample extraction

Extraction in this study was carried out using the maceration method. Powder from pineapple stem as much as 3.2 kg was soaked in jerry cans with n-hexane solvent for 24 hours, then filtered. The maceration process was repeated four times. Then dried. Then the solvent was evaporated using a rotary evaporator so that the crude n-hexane extract of pineapple stump was obtained and then weighed (Juariah & Wati, 2020).

Rejuvenation of test bacteria

Bacteria *S. aureus*, *S. epidermidis* and *Candida albicans* were inoculated onto a slant agar medium by taking one eye of an ose needle aseptically and then inoculated by scraping on a slant agar medium. Furthermore, it was incubated for 24 hours at a temperature of 37^0C (Dewi et al., 2019).

Preparation of bacterial culture suspension

Preparation of bacterial suspensions followed (Ekawati & Azizah, 2017) method with slight modifications. Cultures of *S. aureus* and *S. epidermidis* in the tilted media were taken aseptically as much as one ose, then included in 10 mL of NB (Nutrient Broth) media in different erlenmeyers. Then incubated for 24 hours at a temperature of 37^0C. Bacterial culture contained in NB media was measured OD (optical density) using UV spectroscopy with a wavelength of 600 nm.

Phytochemical test

Phytochemical tests of secondary metabolite content were carried out on pineapple core samples that had been mashed and then used to test for alkaloid,

terpenoid and steroid compounds, saponins, phenolics and flavonoids (Sumiati et al., 2021).

Thin layer chromatography test

Thin layer chromatography test using the method (Hafizah et al., 2024; Runtuboi et al., 2024) with slight modifications. The thick n-hexane extract was diluted slightly using n-hexane solvent and then conducted a KLT test to determine the number of components in the extract. The number of compound components is characterized by the number of stains on the KLT plate. Besides being used to determine the number of components, this KLT test is also used to determine the appropriate eluent for the separation of compounds in n-hexane extract. The KLT test used eluent with a ratio of n-hexane and ethyl acetate (4:1). Research design and method should be clearly defined**.**

Antibacterial activity test

Antibacterial activity test using the method according to Winda et al. (2023) with slight modifications. Bacterial inoculum of *S. aureus* and *S. epidermidis* (OD600nm ~ 0.1) equivalent to 107 CFU/mL was taken as much as 100µL, then put into a test tube containing 25 mL of liquid NA (Nutrient Agar) media and vortexed. Then poured into a petri dish. Wait for the media to solidify. N-hexane extracts with a concentration of 100µg/disc, 75µg/disc and 50µg/disc were dissolved with DMSO solvent and then applied to sterile discs as much as 50µL and pure crystals with a concentration of 40µg/disc, 30µg/disc and 20µg/disc were dissolved with DMSO solvent and then applied to sterile disc paper as much as 20µL. The positive control used is Amoxsan® with a concentration of 75µg / disk and 30µg / disk, each of which is dissolved in DMSO solvent and then applied to sterile disc paper as much as 10µL. The negative control used was DMSO solvent. The dried disc paper was placed on the surface of the test media then incubated at 37µC temperature for 24 hours. The zone of inhibition formed was observed and measured in diameter using a caliper.

Antifungal activity test

Antifungal activity test using the method according to Winarni et al. (2023) with slight modifications. PDA agar medium as much as 25 mL was poured into a Petri dish and then waited until it solidified. *C. albicans* fungal inoculum (OD600nm ~ 0.1) equivalent to 107 CFU/mL was taken as much as 100µL and spread using an L rod onto PDA (Potato Dextrose Agar) media. N-hexane extracts with concentrations of 100µg/disc, 75µg/disc, and 50µg/disc were dissolved with DMSO solvent then applied to sterile discs as much as 50µL and pure crystals with concentrations of 40µg/disc, 30µg/disc and 20µg/disc were dissolved with DMSO solvent then applied to sterile disc paper as much as 20µL. The positive control used is ketoconazole with a concentration of 75µg / disk and 30µg / disk, each of which is dissolved in DMSO solvent and then applied to sterile disc paper as much as 10µL. The negative control used was DMSO solvent. The dried disc paper was placed on the surface of the test media then incubated at 37^0C temperature for 24 hours. The zone of inhibition formed was observed and measured in diameter using a caliper.

Structure elucidation

Compounds that have been purified are carried out structural elucidation using UV and FTIR spectrophotometers and analyzed. This stage is the stage of determining the final compound obtained from the isolation of plant extracts (Riris, 2013).

Result and Discussion

Phytochemical Test Results

In this study the sample used was pineapple core. Pineapple core that has been separated and cleaned is dried at room temperature with the aim of removing the water content contained in the pineapple pith to facilitate the process of withdrawing chemical compounds. The dried pineapple core was then pulverized into powder. This is done to increase the surface area of the sample so that the contact between the solvent and plant particles is greater. Before the maceration process, the powder of pineapple core was tested for phytochemicals first. The results of the phytochemical test showed that the pineapple core was positive for terpenoid and phenolic secondary metabolite compounds. Based on the test results of pineapple core extract and AC compound eh can be seen in Table 1.

Table 1. Phytochemical test results of pineapple core extract

Phytochemical	Reagent	Result	Conclussion
Alkaloid	Mayer	-	No white precipitate formed
	Dragendorff	-	No orange precipitate formed
Terpenoids dan Steroids	Liebermann Burchard	+/-	Brownish red and green solution
Flavonoids	Mg-HCl	-	No reddish orange color formed
Phenolic	$FeCl_3$	+	Formed green precipitate
Saponin	H_2O	-	No foaming

Table 2. Phytochemical test results of Ac-EH compound

Phytochemical	Reagent	Result	Conclussion
Alkaloid	Mayer	-	No white precipitate formed
	Dragendorff	-	No orange precipitate formed
Terpenoids dan Steroids	Liebermann Burchard	+/-	Brownish red and green solution
Flavonoids	Mg-HCl	-	No reddish orange color formed
Phenolic	$FeCl_3$	+	Formed green precipitate
Saponin	H_2O	-	No foaming

Based on the test results that have been carried out, it can be seen that only phenolic compounds are declared positive.

The pure Ac-EH crystals were identified using UV and FTIR spectroscopy and data were compared with existing literature to determine the compounds obtained. The results of the UV spectrum show that the Ac-EH compound gives maximum absorption at wavelengths of 268 nm and 206 nm with absorbance values of 0.174 and 1 with a wavelength range of 200-400 nm. The results of the UV spectrum can be seen in Figure 1.

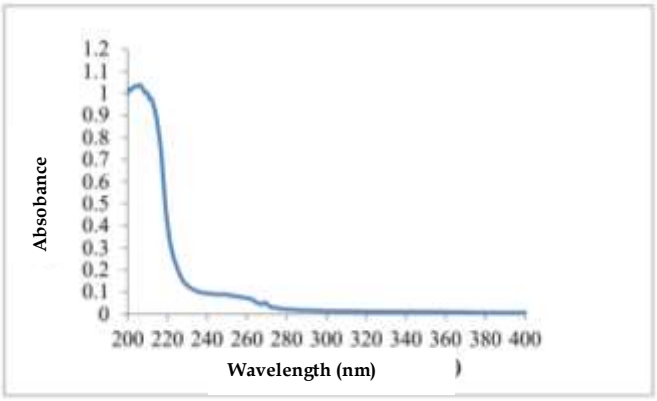

Figure 1. UV spectrum of Ac-EH compound

Ac-EH compounds were characterized using FTIR to see the functional groups present in the compounds with KBr plates at wave numbers 450-4500 cm^{-1}. FTIR results showed that Ac-EH obtained showed absorption at wave number 1047 cm-1 indicating the presence of bond vibrations (C-O). At wave number 1379 cm^{-1}, the absorption band formed indicates the presence of (CH3) bonds, wave number 1465 cm^{-1} indicates the presence of (CH2). Dimethyl groups (two methyl groups on the same carbon) are present at wave numbers 1465 cm^{-1} and 1379 cm^{-1}. This dimethyl geminal group absorption is a typical absorption of steroid and triterpenoid compounds (Fasya et al., 2020). At wave number 2939 cm^{-1} indicates the presence of aliphatic (C-H) groups and at 3309 cm^{-1} absorption indicates the presence of (O-H) bonds which can be seen in Figure 2.

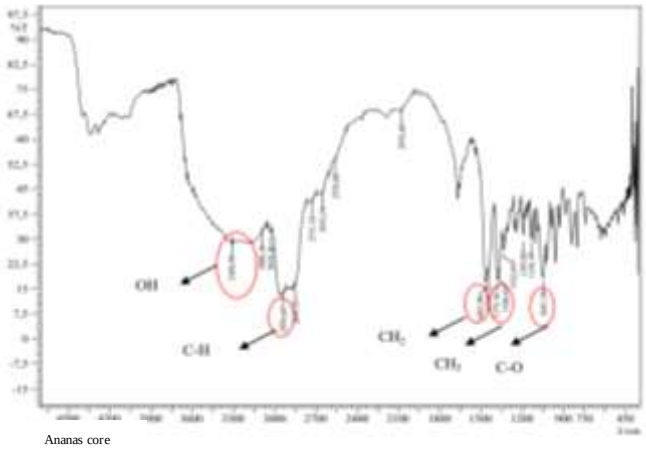

Figure 2. FTIR spectrum of Ac-EH compound

Based on the identification of functional groups in Ac-EH compounds with FTIR, it shows the presence of absorption bands at wave numbers with the appearance of aliphatic C-H stretching vibrations at 2939 cm^{-1} and 2849 cm-1, indicating the possible presence of methyl groups (-CH3) and (-CH2). This data is reinforced by the presence of C-H bending vibrations at wave numbers 1465 cm^{-1} and 1382 cm^{-1} which identify the presence of dimethyl geminal groups as a characteristic of triterpenoid compounds (Sundari, 2020). According to Pamenta (2019) states that one method to elucidate the presence of terpenoid group compounds can be done using FTIR analysis by tracing the presence of functional groups such as the presence of hydroxyl groups (~3400 cm^{-1}) or the presence of oxo groups (1700-1750 cm^{-1}). FTIR analysis of the Ac-EH compound obtained from the isolation of n-hexane extract from pineapple core showed an absorption at 3309 cm^{-1} wave number. This indicates that the Ac-EH compound may contain an O-H stretching functional group.

Thin layer chromatography test

The results of the KLT test show that a good eluent is used, namely n-hexane: ethyl acetate (4: 1) and there are stains of compounds that have been separated Figure 3.

Figure 3. Thin layer chromatography test (n-heksana : etil asetat, 4 : 1)

Antibacterial activity test

Antibacterial activity test was conducted against two types of pathogenic bacteria, namely *S. aureus* and *S. epidermidis*. The results of the antibacterial activity test showed that the n-hexane extract and Ac-EH compound had moderate antibacterial activity. The results of the n-hexane extract antibacterial activity test can be seen in Table 3 and the results of the Ac-EH compound antibacterial activity test can be seen in Table 4.

Table 3. Diameter of zone of inhibition of n-hexane extract of pineapple core

Sample Concentration (µg/disck)	Inhibition Zone Diameter (mm)	
	S. aureus	S. epidermidis
40	8.71	9.81
30	8.44	9.51
20	8.12	9.13
Amoxsan® (75µg/disk)	17.42	19.71
Negative control	-	-

Table 4. Inhibition zone diameter of Ac-EH compound

Sample Concentration (µg/disck)	Inhibition Zone Diameter (mm)	
	S. aureus	S. epidermidis
40	9.41	9.13
30	8.91	8.71
20	8.52	8.32
Amoxsan® (75µg/disk)	18.21	18.81
Negative control	-	-

The results showed that the samples had antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*, with the largest inhibition zone at a concentration of 40 µg/disc, which was 8.71 mm against *S. aureus* and 9.81 mm against *S. epidermidis*. As the concentration decreased, the zone of inhibition decreased, indicating a concentration-dependent relationship. Compared to the positive control Amoxsan® (75 µg/disc), which produced a zone of inhibition of 17.42 mm against *S. aureus* and 19.71 mm against *S. epidermidis*, the sample showed lower effectiveness. The absence of inhibition zone in the negative control indicates that the medium without active substance has no antibacterial effect. These results are consistent with recent studies showing that the antibacterial effectiveness of natural compounds depends on their concentration and chemical composition (Movahed et al., 2021).

The difference in inhibition against *S. epidermidis* and *S. aureus* can be caused by differences in cell wall structure, where *S. aureus* has stronger defense mechanisms such as the production of beta-lactamases that can inhibit the action of antibiotics (Guo et al., 2020). Recent studies have also shown that plant extracts containing flavonoids and phenolics have varying antibacterial activity depending on the structure of the active compound and the type of target bacteria (Liu et al., 2022). Therefore, although the samples show potential as antibacterial agents, their effectiveness still needs to be improved through formulation optimization or combination with other more potent compounds (Wang et al., 2023). Recent studies have also shown that plant extracts with flavonoids and phenolic content have varying antibacterial activity depending on the structure of the active compounds and the type of target bacteria (Liu et al., 2022)

The other research also showed that red ginger (*Zingiber officinale* var. rubrum) and black turmeric (*Curcuma caesia*) extracts have significant antibacterial potential against various pathogenic bacteria, including *Klebsiella pneumoniae* and bacteria that cause nosocomial infections (Juariah et al. 2023). In addition, further studies found that ethanol extract of red ginger can inhibit the growth of pathogenic bacteria through the mechanism of cell wall inhibition and the production of secondary metabolites that act as antibacterial agents (Juariah, et al., 2023). Another study also revealed that *Curcuma caesia* has phytochemical properties that contribute to significant antibacterial and antioxidant activities (Kartini et al., 2023).

Antifungal activity test

Antifungal activity test was conducted against pathogenic fungi, namely *C. albicans*. The results of the antifungal activity test showed that the n-hexane extract and Ac-EH compound were not active against *C. albicans* fungi. The results of the n-hexane extract antifungal activity test can be seen in Table 5 and the results of the Ac-EH compound antifungal test can be seen in Table 6.

Table 5. Diameter of zone of inhibition of n-hexane extract of pineapple core

Sample Concentration (µg/Disk)	Inhibition Zone Diameter (mm)
	C. albicans
40	-
30	-
20	-
Ketokonazole(75µg/disk)	15.41
Negative Control	-

Table 6. Inhibition zone diameter of Ac-EH compound

Sample Concentration (µg/Disk)	Inhibition Zone Diameter (mm)
	C. albicans
40	-
30	-
20	-
Ketokonazole(75µg/disk)	12.22
Negative Control	-

The results showed that samples with concentrations of 40, 30, and 20 µg/disk did not produce a zone of inhibition against *Candida albicans*, while the positive control ketoconazole (75 µg/disk) produced a zone of inhibition of 15.41 mm. The absence of inhibition zone in the samples indicates that the tested compounds do not have significant antifungal activity or that the concentration used is not effective enough. Ketoconazole works by inhibiting the biosynthesis of ergosterol, a major component of fungal cell membranes, leading to membrane disruption and cell death (Guo et al., 2020). Previous studies have shown that the antifungal effectiveness of natural compounds depends on their chemical structure and the dose used, and can be enhanced through combination with other compounds or formulation modification (Movahed et al., 2021; Wang et al., 2023). A recent study found that mixed ethanol extracts of red ginger (*Zingiber officinale* var. rubrum) and black turmeric (*Curcuma caesia*) have significant antifungal activity against *Candida albicans*, which is thought to be related to the content of phenolic compounds and flavonoids that play a role in damaging the integrity of the fungal cell membrane (Juariah et al., 2024).

In addition, research on red galangal rhizome extract (*Alpinia purpurata* K. Schum) also showed its effectiveness in inhibiting the growth of *C. albicans*, indicating that active compounds such as phenolics and essential oils play a role in its antifungal activity (Juariah, et al., 2023). Another study examining pineapple (*Ananas comosus* L. Mer) core extract also reported that bromelain enzyme content can inhibit the growth of *C. albicans* by disrupting cellular adhesion and degradation of fungal cell wall proteins (Juariah et al., 2020). Therefore, the antifungal effectiveness of natural compounds may depend on their bioactive content, and combination or dose optimization may be a potential strategy to enhance their antifungal activity.

In addition, the study of compound Ac-EH showed that at concentrations of 40, 30, and 20 µg/disk, no inhibition zone was formed against *C. albicans*. This is similar to the previous results, indicating that these compounds do not have significant antifungal activity in the concentrations tested. In comparison, the positive control ketoconazole (75 µg/disk) produced a zone of inhibition of 12.22 mm, which was smaller than the previous study that showed a zone of inhibition of 15.41 mm. This difference could be due to variations in the test method, the *C. albicans* strain used, or environmental conditions during testing (Guo et al., 2020). Some studies show that the antifungal effectiveness of a compound can also be affected by interactions with the culture media environment as well as pH and temperature factors (Wang et al., 2023). Therefore, concentration optimization and combination with other compounds need to be considered to increase the antifungal potential of Ac-EH against *C. albicans*.

Conclusion

Based on the results of the study conducted on the isolation of compounds and n-hexane extracts from pineapple tubers, it can be concluded that: 1). The results of the isolation of secondary metabolites from n-hexane extracts of pineapple tubers obtained the compound Ac-EH which is suspected to be a euphorbol compound with a weight of 15.9 mg. 2). The results of the antimicrobial activity test carried out by the diffusion method showed that the n-hexane extract and the compound Ac-EH from pineapple tubers were able to inhibit *S. aureus* and *S. epidermidis* bacteria and had an inhibition zone diameter that was not too far so that the smallest concentration was able to inhibit the bacteria tested while in *C. albicans* fungus it was not active. The n-hexane extract inhibited *S. aureus* by 9.1 mm, 9.5 mm, and 9.8 mm and *S. epidermidis* by 8.1 mm, 8.4 mm, and 8.7 mm. The Ac-EH compound inhibited *S. aureus* by 8.5 mm, 8.9 mm, and 9.4 mm and *S. epidermidis* by 8.3 mm, 8.7 mm, and 9.1 mm. This can be stated that the antibacterial ability of pineapple corm extract is at a moderate level. 3). The results of the characterization of the pure compound Ac-EH using a UV spectrophotometer showed maximum absorption at wavelengths of 268 nm and 206 nm. FTIR spectrophotometer shows absorption at wave numbers 1047 cm-1 (C-O), 1379 cm-1 (CH3), 1465 cm-1 (CH2), 2939 cm-1 (C-H) aliphatic and 3309 cm-1 (OH).

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Author Contributions

In this study, there are several authors who contributed, including Conceptualization, S, R; methodology, S, R; validation, S, R and N; formal analysis, S, R; investigation, S, R; resources S, R and N; data curation, S, R and N: writing—original draft preparation, S.R and N.; writing—review and editing, S, N.: visualization, S and N. All authors have read and approved the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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