

An In Vitro Approach: Antibacterial Activity of *Sansevieria trifasciata* Prain. Leaves with Chemometric Analysis

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Abstract

Exploration the antibacterial activity of *S. trifasciata* Prain. is still limited, therefore this study aims to assess the antibacterial activity of extracts and ethyl acetate fractions of *Sansevieria trifasciata* Prain. The *S. trifasciata* leaves was macerated with ethanol 96%, then fractionated using the trituration method with ethyl acetate. The treatment group was divided into positive control group (PC) using ciprofloxacin, negative control (NC) using DMSO, extract, ethyl acetate fraction 5% (ET5%), 10% (ET10%), 20% (ET20%), 40% (ET40 %). Data were analyzed statistically by ANOVA and chemometrically with PCA. The inhibition zone for *S. aureus* bacteria in each sample is 26.69; 1.40; 23.32; 2.82; 6.23; 11.11; 20.15 mm, respectively, *E. coli* is 26.65; 0.63; 22.65; 3.61; 7.11; 11.44; 21.15 mm respectively, *P. aeruginosa* is 27.40; 0.00; 23.23; 2.74; 7.03; 11.69; 21.36 mm respectively. Percent inhibition of extract, ET5%, ET10%, ET20%, ET40% on *S. aureus* bacteria is 82.16; 5.31; 18.12; 36.39; 70.38% respectively, *E. coli* is 82.67; 11.13; 24.31; 40.56; 76.99% respectively, *P. aeruginosa* 84.85; 10.01; 25.65; 42.68; 77.98% respectively. Extract and ethyl acetate fraction have significant potential as antibacterial ($p < 0.05$). The results of PCA chemometric analysis showed that the extract and ET40% had similar inhibition zone area to the positive control ciprofloxacin. The extract and the ethyl acetate fraction 40% are promising for development as antibacterials.

Keywords: *Sansevieria trifasciata* Prain., chemometric, bacterial

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1 Introduction

The Indonesian sustainable development goals acceleration program, particularly in the health sector, necessitates the rigorous management of diseases with high mortality rates, infectious disease being one of them [1]. According to research on the prevalence of infectious diseases, the majority of infections are caused by microbes. According to Riskesda data from Indonesia, the most common prevalence of bacterial infectious diseases includes diarrhea which causes 14.5% of deaths, pneumonia which is 9.5% in children [2]. WHO estimates that the infection cases will increase to 10 million deaths in 2050 and 4.7 million of them are Asian residents. According to existing research, there are 33 distinct bacterial species that have been identified as causative agents of fatal infections worldwide, which bacteria caused the largest number of deaths are *Staphylococcus aureus* with more than 1 million deaths, *Escherichia coli*, and *Pseudomonas aeruginosa* are giving cases of death of more than 500 thousand [3]. *Staphylococcus aureus* and *Escherichia coli* bacteria are commonly found as commensal microorganisms within the human body, but under certain circumstances, these bacteria can transform into significant zoonotic pathogens leading cause diseases [4]. Multiple research investigations conducted in Indonesia have provided evidence indicating the presence of bacteria in the currently available food and beverage products [5]–[7]. The interesting thing is these bacteria are also found in the form of bacteria that are resistant to antibiotics [5], [8]. This is a significant issue, as the treatment of diseases caused by antibiotic-resistant bacteria is becoming increasingly complex, as doctors must match antibiotics that patients can still use with the pattern of resistant bacteria. Consequently, current scientific investigations are underway to discover novel generations of antibiotics in

order to prevent occurrences of antibiotic resistance. These efforts involve studying the potential of natural compounds as potential therapeutic agents.

The *Sansevieria trifasciata* Prain is known as a natural component that has been historically employed in medicinal applications, such as antibacterial [9], antimalarial [10], antifungal [11], prevents uric acid excretion by inhibiting xanthine oxide [12], antiulcerative [13], antidiabetic [14], treatment of corn [15], anthelmintic [16], antiallergic and anti-anaphylactic [17], antioxidant and antimutagenic [18]. The LC-MS/MS analysis revealed the presence of several bioactive compounds, namely 1-acetyl- β -carboline, oliveramine, trichosanic acid, (2s), 3',4'-methylenedioxy-5,7 dimethoxyflavane, methyl pirophaeporbid A, methyl gallate, and a digiprolactone compound known for its anti-alopecia properties [19], [20]. Furthermore, it has been reported that the substances 1,2-(dipalmitoyl)-3-O- β -D-galactopyranosyl glycerol, Sansevierigenin, and Spirosta-5,25(27)dien-1b,3b-diol-1-O-a-L-rhamno pyranosyl-(1, 2)-a-L-arabinopyranoside exhibit anti-alopecia efficacy through inhibiting the androgen receptor pathway [21]. Some studies indicating that *S. trifasciata* Prain. have the potential to be further developed as an antibacterial agent [22]. Recent study have demonstrated that the ethanolic extract has inhibitory effects on the growth of *E. coli* and *S. aureus* bacteria, as evidenced by minimum inhibitory concentration (MIC) values of 50 mg/mL and 25 mg/mL, respectively [9]. The growth of *Pseudomonas aeruginosa* bacteria can be inhibited by the ethanol extract and fraction, which the neophytadiene compound thought to play a role in this inhibitory effect of bacterial growth [23]. Unfortunately, published data on the antibacterial activity of this plant are still

severely restricted, both in terms of sample type and bacterial species.

The ethyl acetate fraction of *S. trifasciata* is believed to possess antibacterial properties due to its chemical composition. Methyl gallate, a chemical detected in the ethyl acetate fraction of *S. trifasciata* using LC-MS-MS analysis, has exhibited growth inhibiting properties on *S. Gallinarum*, *S. Typhimurium*, *S. Enteritidis*, *S. Typhi*, *S. Paratyphi A* [24], *R. solanacearum* [25], *E. coli* and *P. aeruginosa* [26]. Nevertheless, there is a lack of prior research on the antibacterial properties of the ethyl acetate fraction using the well diffusion method against the *S. aureus* bacteria. Furthermore, the existing approach for statistical analysis of antibacterial assay data in prior studies is constrained to detecting significant differences in the standard error. However, there is a dearth of research employing chemometric Principal Component Analysis (PCA) to examine classifying patterns based on similarity in the inhibition zone data of *S. trifasciata*. This novel method presents an opportunity to explore uncharted territory in this field. The data assists in determining the sample that exhibits the highest level of therapeutic efficacy. Therefore, in this study we carried out the antibacterial assay of extract and ethyl acetate fraction from *S. trifasciata* Prain. leaves against *E.coli*, *S.aureus*, *P. aeruginosa* bacteria using well diffusion method to obtain the inhibition zone.

2 Methods

2.1 Maceration and fractionation

Sansevieria trifasciata Prain. Leaves were collected from Unaaha Konta Kendari District. Samples were determined at the Sekolah Ilmu dan Teknologi Hayati Laboratory, Institut Teknologi Bandung. The *S. trifasciata* Prain. leaves washed, chopped and dried in the oven (B-One®) (60°C), grind to obtain the dry simplicia powder. Then, the simplicia was macerated with ethanol 96%, and the solvent being changed every 24 hours. The macerate was filtered, collected, and concentrated using a rotary vacuum evaporator (Buchi®) to obtain the crued extract. The extract (75.03 g) was then fractionated using the trituration method. The extract was placed in a mortar and the n-hexane solvent was, and crushed slowly to remove the

ballast [22]. The hexane filtrate is collected, while the residue is added with ethyl acetate solvent, crushed, the filtrate is collected as an ethyl acetate fraction. The ethyl acetate phytrate was concentrated using a rotary vacuum evaporator (Buchi®) to obtain the crude ethyl acetate fraction, and the yield of fraction was calculated.

2.2 Phytochemical screening

Screening for phytochemicals analysis was carried out on extract samples and ethyl acetate fractions with a slightly modified method [27] :

2.2.1 Flavonoids

Each sample (1 mL) was put into a test tube, then 2 drops of HCl were added and shaken vigorously. Then, magnesium (Mg) powder is added into the tube. Samples positive for containing flavonoids if the color of sampel changed to orange, pink or dark red and yellow.

2.2.2 Alkaloids

Sample (1 mL) was added with chloroform and NH₃. Then heated over a water handler. A drop of H₂SO₄ was added into tube then dragendroff's reagent. A positive sample contains alkaloids if there were orange or red or red-brown precipitates.

2.2.3 Saponin

Sample (1 mL) was placed in a reaction tube and 5 mL of hot water was added, then the tube was shaken. After shaking the tube for 10 minutes, leave it for 10 minutes. The positive result if there was a foam and doesn't disappear during shaking.

2.2.4 Tannin

The prepared sample solution was added with potassium acetate and 1 ml of 1 ppm FeCl₃ solution then the changes were observed. A sample was positive if it produced a strong green, purple, red and blue or black color.

2.2.5 Terpenoids

Samples was added with 0.5 mL of anhydrous acetic acid then added with 2 mL of H₂SO₄. A positive test for steroids was indicated by the formation of blue and green colors. The formation of orange, purple and golden yellow colors indicated a positive test for triterpenoids.

2.3 Media and Organism

The bacteria used in the well diffusion assay were obtained from the Faculty of Medicine, Halu Oleo University, namely *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853. The media used in bacterial rejuvenation and testing were nutrient agar (NA). Media preparation was carried out by dissolving nutrient agar (28 g) in distilled water, then heating, stirring until dissolved, then sterilizing in an autoclave (Daihan WACS®).

2.4 Sample preparation

The samples used consisted of the positive control group (PC) which was given ciprofloxacin tablets (Bernofarm®) (50µg), the negative control (NC) was given DMSO (Merck®) (10%), the treatment group was the ethyl acetate fraction of *S. trifasciata* Prain. with varying concentrations of 5% (ET5%), 10% (ET10%), 20% (ET20%), 40% (ET40%). The variation of concentration from ethyl acetate fraction was made using DMSO solvent. Samples were prepared by weighing ethyl acetate fractions about 25, 50, 100, and 200 mg, and dissolving each in 500 µL DMSO to produce ET5%, 10%, 20%, 40%, respectively.

2.5 Antibacterial activity

The antibacterial activity was done with well diffusion method. The antibacterial assay process was carried out aseptically in a class II biosafety cabinet (Prolab®). Previously, the bacterial suspension was adjusted to 1×10⁸ CFU/mL using McFarland 0.5 in sterile saline 0.9%. The liquid nutrient agar medium (15 mL) is placed in a sterile Petri dish, leveled, and allowed to stand until it solidifies. Then added on top of the solid media, the suspension of each test bacteria (1 mL), spread evenly. NA media (7 mL) was added as a second layer, left to solidify. Six wells were made using a 7 mm diameter probe, then the sample solution (PC, NC, ET5%, ET10%, ET20%, ET40%) was added to each well about 50 µL, respectively, the petri dish was closed, and incubated (Mettler®) for 24 hours at 37°C. The inhibition zone formed was observed and measured using a screw micrometer. Antibacterial activity is expressed as the diameter of the inhibition zone, and the percentage of inhibition is calculated as equation 1 [28].

$$= \frac{\text{Inhibition zone of sample} - \text{inhibition zone of solvent or negative control}}{\text{inhibition zone of positive control}} \times 100\% \quad (\text{Equation 1})$$

2.6 Data Analysis

The diameter of the inhibition zone was analyzed statistically using the IBM Statistics® SPSS version 24 application using ANOVA and LSD posthoc with statistical significance at a 95% confidence level ($p < 0.05$) were performed to identify group differences to determine its potential and effectiveness as an antibacterial. Multivariate mapping patterns of treatment groups were analyzed using Principal Component Analysis (PCA) using the Minitab® version 17 application.

3 Results and Discussions

3.1 Fractionation and Phytochemical Screening

Simplisia of *S. trifasciata* Prain. macerated and fractionated using solvents with varying polarities from the non-polar solvent n-Hexane followed by the polar solvent ethyl acetate. In this study, the ethyl acetate fraction was used for further testing, but previously the percent yield of fraction was calculated. The calculation result of the percent yield of the ethyl acetate fraction from an extract weight of 75.03 grams was 14.88%. This indicates that from the fractionation process only 14.88% of the compound can be dissolved in ethyl acetate.

Table 1. The results of phytochemical screening of *S. trifasciata* Prain. leaves extracts and ethyl acetate fractions

Chemical Compound	Sample	Result	Information
Alkaloids	Ethanollic extract	+	red precipitate form
	Ethyl acetate fraction	+	Precipitate form
Saponin	Ethanollic extract	+	Foam
	Ethyl acetate fraction	+	Foam
Terpenoids	Ethanollic extract	+	Brown precipitate form
	Ethyl acetate fraction	+	Brown precipitate form
Flavonoids	Ethanollic extract	+	Orange precipitate form
	Ethyl acetate fraction	+	Orange precipitate form
Tannin	Ethanollic extract	+	Precipitate
	Ethyl acetate fraction	+	Black precipitate

The results of phytochemical screening of samples, both extracts and fractions, showed *S.*

trifasciata Prain leaves. contains alkaloids, terpenoids, flavonoids, tannins and saponins (Table 1). This is in line with previously reported research [29].

3.2 Antibacterial activity

Sansevieria trifasciata Prain. exhibits potential as a botanical candidate for antibacterial development. The well diffusion method was employed to conduct an antibacterial test on extracts and fractions of

S.trifasciata in the present investigation. The obtained results encompass the measurement of the inhibition zone's area, the percentage of inhibition, and the grouping profile of the samples, which is determined by the similarity of the inhibition zone's area in multivariate analysis using PCA. The bacterial strains employed in this investigation consisted of gram-positive bacteria *S. aureus*, and the gram-negative bacteria, specifically *E. coli* and *P. aeruginosa*.

Table 2. The results of antibacterial activity assays on the extract and ethyl acetate fraction of *S. trifasciata* Prain. (data presented as inhibition zone in millimetres (mm)).

Sample	Inhibition zone of bacterial growth (mm)								
	<i>Streptococcus aureus</i>			<i>Escherichia coli</i>			<i>Pseudomonas aeruginosa</i>		
Extract	23.32	± 0.52	^{a,b}	22.65	± 0.75	^{a,b}	23.23	± 0.95	^{a,b}
ET5%	2.82	± 0.38	^a	3.61	± 0.29	^{a,b}	2.74	± 0.13	^{a,b}
ET10%	6.23	± 0.14	^{a,b}	7.11	± 0.51	^{a,b}	7.03	± 0.46	^{a,b}
ET20%	11.11	± 0.19	^{a,b}	11.44	± 0.19	^{a,b}	11.69	± 0.19	^{a,b}
ET40%	20.15	± 1.98	^{a,b}	21.15	± 0.25	^{a,b}	21.36	± 0.52	^{a,b}
Positive control	26.69	± 0.62	^b	26.65	± 0.66	^b	27.40	± 0.66	^b
Negative control	1.40	± 0.33	^a	0.63	± 0.38	^a	0.00	± 0.00	^a

The results of the one-way anova post-hoc LSD analysis are displayed as an if : ^asignificant difference ($p \leq 0.05$) between the positive control (Ciprofloxacin); otherwise ^b significantly different ($p \leq 0.05$) to the negative control (DMSO).

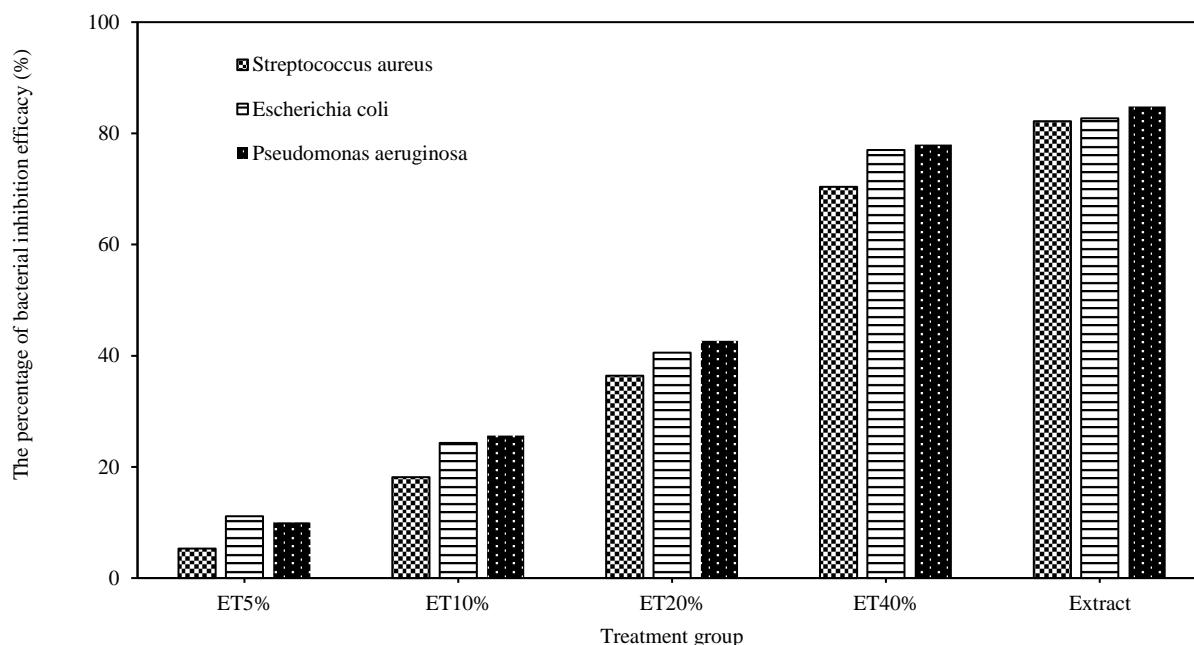


Figure 1. The percentage of bacterial inhibition after treatment. The experimental groups were comprised of fractions of ethyl acetate at concentrations of 5% (ET5%), 10% (ET10), 20% (ET20%), and 40% (ET40%), and extracts of *S.trifasciata* Prain. against *S. aureus*, *E. coli*, and *P. aeruginosa* bacteria.

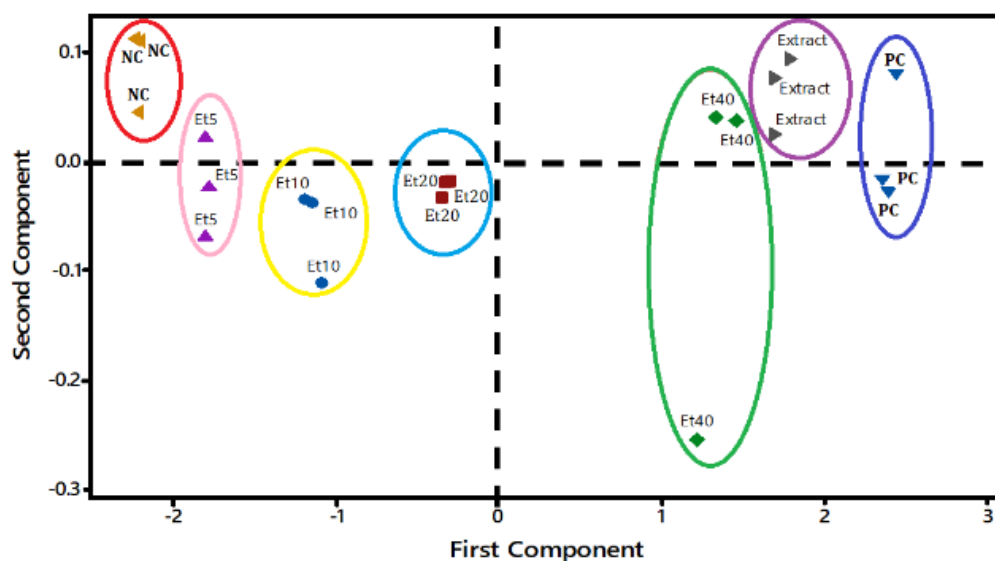


Figure 2. Profile score plot from PCA chemometric analysis of the inhibition zone area for each treatment group. The areas of bacterial growth inhibition in the negative control, positive control, extract, and the ethyl acetate (ET) fractions group of 5%, 10%, 20% and 40% are shown in red, blue, purple, orange, yellow, light blue and green circles in a row. The ET 40% group and the extract were in the same quadrant with the positive control. Meanwhile ET5%, 10%, and 20% are in the same quadrant with the negative control.

The results of the antibacterial assay (Table 2) prove that both the ethyl acetate extract and fraction have strong inhibitory effects on the growth of the three bacterial strains ($p < 0.05$), with the highest percentage of growth inhibition seen (Figure 1). The inhibitory effect of the extract and the 40% ethyl acetate fraction is shown to be quite significant, as indicated by the inhibitory zone diameter of ≥ 21 mm. The samples containing ethyl acetate at concentrations of 5%, 10%, and 20% exhibited inhibitory intensities classified as moderate, medium, and high, respectively [30]. The posthoc LSD statistical analysis (Table 2) revealed significant differences between each group in comparison to the positive control. This observation suggests that there is a disparity in the efficacy between the test sample and the control. Specifically, the positive control demonstrates more effective results compared to the extract and ethyl acetate fraction in terms of preventing bacterial growth.

The results of analyzing the percentage of inhibition indicate the antibacterial activity of each sample. The percent inhibition of extract, ET5%, ET10%, ET20%, and ET40% against *S. aureus* bacteria is 82.16; 5.31; 18.12; 36.39; 70.38; *E. coli* is 82.67; 11.13; 24.31; 40.56; 76.99%; and *P. aeruginosa* is 84.85; 10.01;

25.65; 42.75; 77.98% (Figure 1). These results indicate that the percent inhibition of the extract is greater than that of the other samples, which are ET40%, ET20%, ET10%, and ET5%, respectively. This is inline with the results of the inhibitory zone diameter, which confirms the extract possesses a strong inhibitory effect.

PCA chemometrics was employed to analyse the similarity patterns of the inhibitory zone of the bacteria in each treatment group. Multivariate analysis utilising Principal Component Analysis (PCA) is a suitable approach for the comparison of detailed datasets. This methodology is particularly applicable when the data contains a combination of pharmacological activity and therapeutic/phytomedicine groups, thereby enabling the identification of active fractions or compounds that exhibit promising prospects for subsequent development [31]. The chemometric analysis findings demonstrate the clustering of data according to the similarity seen in the inhibition zones of each treatment group. The profile score plot (Figure 2) illustrates the division of the data into two distinct regions. The group and ethyl acetate fraction of *S. trifasciata* Prain were isolated. The observed proportion of 40% aligns with the positive control group, but the ethyl acetate

fractions of 5%, 10%, and 20% are situated in proximity to the negative control group. This clarified that the extract and 40% fraction exhibit comparable characteristics to the positive control group in their ability to inhibit bacterial growth, hence indicating their potential for further development as antibacterial medication. Different occurrences occurred within the fractions of 5%, 10%, and 20% in the area of the negative control, signifying limited suppression of bacterial growth and proximity to untreated conditions.

The relationship between the concentration of the ethyl acetate fraction of *S.trifasciata* Prain. used in antibacterial tests and the resulting inhibitory zone is directly correlated. The higher the concentration employed, the greater the abundance of active chemicals present [32]. Several active compounds from *S.trifasciata* Prain. has been successfully identified, one of which is a derivative pyridone alkaloid (5-methyl-11-(2-oxopyridin-1(2H)-yl)undecaneperoxoic acid) which is known to inhibit bacterial growth through an inhibitory mechanism on β -ketoacyl-ACP synthase and TyrRS [22]. In addition, the terpenoid neophytadiene derivative compounds that have been identified are thought to have antibacterial activity [33]. The antibacterial activity of the ethyl acetate fraction in *S.trifasciata* Prain. is believed to be attributed to the presence of those chemical compound.

4 Conclusions

The extract and ethyl acetate fractions have potential for developing as antibacterial agents. Specifically, the extract and the 40% ethyl acetate fraction exhibit efficacy comparable to that of ciprofloxacin, as determined by principal component analysis (PCA).

5 Declarations

5.1 Acknowledgment

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5.3 Authors Contributions

Henny Kasmawati: Coordinator of sampel preparation and antibacterial assay in the laboratory, collecting and reviewing sample literature and methods, analyzing research data using SPSS, writing the article, checking article plagiarism, submitting article. Nurramadhani A. Sida: analysis of the data using chemometrics, compiling research data, write the article manuscripts, templating articles. Wa Ode Dian Indrayanti: carrying out technical research implementation, preparing research results reports, writing article manuscripts. Arman Rusman: compiling research data, write the article manuscripts, templating articles

5.4 Conflict of Interest

The authors declare no conflict of interest

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