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Tradescantia pallida extract inhibits biofilm formation in *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is capable of biofilm formation. In this study, we investigated the effects of aqueous *Tradescantia pallida* extract on *Pseudomonas aeruginosa* growth and biofilm formation. Aqueous *Tradescantia pallida* extracts significantly inhibited both bacterial growth and biofilm formation. However, methanolic *Tradescantia pallida* extracts inhibited neither. Aqueous *Tradescantia pallida* extracts were deactivated by heating but were not deactivated by light exposure. The ingredients retained the inhibitory effect on the bacterial growth and biofilm formation after ultrafiltration of aqueous *Tradescantia pallida* extract. Furthermore, polyphenol-rich *Tradescantia pallida* extracts inhibited bacterial growth, thus, polyphenols are possible to be an active ingredient.

We observed the biofilm by scanning electron microscopy, and quantitative and qualitative differences in the biofilm and cells morphology. Interestingly, the biofilm treated aqueous *Tradescantia pallida* extracts remained premature. We postulated that premature biofilm formation was due to the inhibition of swarming motility. Indeed, aqueous *Tradescantia pallida* extracts inhibited swarming motility. These results demonstrate that *Peudomonas aeruginosa* growth and biofilm formation are inhibited by aqueous *Tradescantia pallida* extracts.

Keywords: *Pseudomonas aeruginosa*, *Tradescantia pallida*, bacterial growth, biofilm formation

Abbreviations:
PAO1: aeruginosa ATCC15692
Tp: *Tradescantia pallida*
BHI-B: Brain Heart Infusion broth
OD: optical density
PBS: phosphate buffered saline
EPS: extracellular polysaccharides

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INTRODUCTION

Pseudomonas aeruginosa is a gram-negative, aerobic bacterium that is ubiquitous in the environment. It can switch between growth as planktonic cells and as a biofilm.[1] Biofilms can be simply and broadly defined as communities of microorganisms that are attached to a surface.[2] Numerous bacterial species form biofilms, and research regarding biofilms has shown them to be complex and diverse.[3]

Biofilms are formed from individual planktonic cells in a highly regulated developmental process. Planktonic cells are considered to initiate interactions with a surface in response to various signals, including the nutritional status of the environment.[4] Bacteria are able to undergo different types of motility that vary depending on the bacterium. Numerous planktonic organisms are able to initially colonize a surface by utilizing a flagellum to swim toward the surface and bacterial adhesins, such as type-IV pili and flagella, to attach to the surface.[5] Once the bacteria are attached, combinations of specific surface-associated motilities, replication (clonal growth), and/or recruitment of additional planktonic bacteria lead to the formation of bacterial aggregates called microcolonies, which can subsequently lead to mature biofilm development.[5]

Proanthocyanidins, a type of tannins derived from cranberries, have been reported to interfere with bacterial adhesions.[6] *Tradescantia pallida* (Tp) has already reported its antibacterial activities against gram-positive and gram-negative bacteria.[7] Thus, we screened several plant extracts containing polyphenols for anti-biofilm activity. Among the extracts, aqueous extracts of Tp leaves showed significant anti-biofilm and non-significant growth inhibition activities (data not shown). This study aimed to investigate the inhibitory effect of Tp extract on the growth and biofilm formation of *P. aeruginosa*.

MATERIALS AND METHODS

Bacterial strain and growth conditions

The bacterial strain used in this study was *P. aeruginosa* PAO1 (ATCC15692; ATCC, Manassas, VA, USA). This strain was cultured in Brain Heart Infusion broth (BHI-B; Eiken chemical, Tokyo, Japan) at 37°C for 18–22 hours.

Preparation of aqueous Tp extracts

Sliced Tp leaves (1 g) were used for extraction using distilled water (12.5 mL) at 37°C with shaking for three days. The solvent was exchanged every 24 hours. The total mixture was then filtered through membrane filters (Acrodisc 32 mm Syringe Filter with 0.45 μm Supor membrane; PALL Life Sciences, Port Washington, NY, USA).

Preparation of methanolic Tp extracts

Sliced Tp leaves (1 g) were used for extraction using 100% methanol (10 mL; Kanto chemical, Tokyo, Japan) at 37°C with shaking for three days. The solvent was exchanged every 24 hours.

Preparation of polyphenol-rich Tp extracts

The polyphenol-rich fraction was extracted from Tp following a protocol published by Konaté et al,[8] with some modifications. Sliced Tp leaves (1 g) were extracted using aqueous acetone (80%, v/v; 10 mL; FUJIFILM Wako Pure Chemical, Osaka, Japan) at 37°C with shaking for 24 hours. The acetone was then evaporated, and the remaining residue was dissolved with distilled water.

Heat exposure assay

2.7% aqueous Tp extracts were heated in constant-temperature water bath at either 60 °C or 80°C, with shaking for six hours.

Light exposure assay

2.7% aqueous Tp extracts were exposured to fluorescent light about 1000–1500 lux for three days.

Ultrafiltration assay

Aqueous Tp extracts was fractionated using an ultrafiltration membrane Amicon® Ultra (nominal molecular weight limit (NMWL) of 10 kDa, Merck Millipore Ltd., Germany) into two molecular weight ranges (< 10 kDa and > 10 kDa). The 2.7% aqueous Tp extracts were added to 12 mL to the filter device, and the device was spun at 5000 × g for 30 minutes. The fraction > 10 kDa was collected in the filter device, and the fraction < 10 kDa was collected in the centrifuge tube.

Bacterial growth assay

The effect of Tp extracts on the growth of *P. aeruginosa* PAO1 in a liquid culture was measured using a protocol based on the protocol published by Matsunaga et al.[9] Growth assays were performed using BHI-B. Aliquots of Tp extracts were inoculated into the wells of a 96-well microtiter plate (polystyrene; AS ONE, Osaka, Japan). Subsequently, an aliquot from a bacterial broth culture (BHI-B, 37°C, 18–22 hours) was inoculated into each well. The plates were covered and incubated at 37°C for 18–22 hours. Bacterial growth was monitored by measuring the optical density at 600 nm (OD_{600}) using a spectrophotometer. The starting OD_{600} of the liquid culture for the bacterial growth assay was 0.05.

Biofilm formation assay

Biofilm formation was assayed by the ability of cells to adhere to the wells of a 96-well microtiter polystyrene dish, using a modified version of a previously reported protocol by O' Toole et al.[10] Briefly, bacteria grown overnight in BHI-B were suspended in fresh BHI, and then diluted to an OD_{600} = 0.05. Aliquots of the bacterial suspension (100 µL) were then inoculated into a 96-well polystyrene plate. After incubation for 18–22 hours at 37°C, the media and unattached bacterial cells were removed from the wells, and the plates were rinsed three times with phosphate buffered saline (pH 7.4; PBS). Next, the wells were stained by adding crystal violet (0.1% w/v; 130 µL) for 20 minutes. The plates were then rinsed with distilled water, and the amount of attached material was quantified by solubilizing the crystal violet dye in 95% ethanol (150 µL). The optical density was measured at 595 nm (OD_{595}) using a spectrophotometer.

Imaging of the samples using scanning electron microscope

PAO1 biofilms were visualized by scanning electron microscopy (SEM; JCM-6000 plus, JEOL, Tokyo, Japan). Bacteria grown overnight in BHI-B were suspended in fresh BHI, and then diluted to an OD_{600} = 0.05. Aliquots of the bacterial suspension (1000 µL) with 0.27% aqueous Tp extracts were then inoculated on sterile membrane filter (0.45µm; Merck KGaA, Darmstadt, Germany) into a 24-well polystyrene plate (Corning Inc., Corning, NY, USA) for four days. The medium was exchanged every 24 hours. Then, the membrane filters were rinsed two times with PBS, fixed by 15% neutral buffered formalin (pH 7.4), re-fixed by 1% osmium tetroxide (TAAB, Aldermaston, Berks, England), dehydrated and replaced with tert-butyl alcohol (FUJIFILM Wako Pure Chemical). Tert-butyl alcohol was sublimed, and the membrane filters were coated of thin

platinum layer using deposition apparatus (JFC-1600; JEOL).

Swarming motility assay

Swarming motility assays were performed 0.5% LB agar plate (LB Broth with agar (Miller); Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) in a petri dish (polystyrene, 90 mm; AS ONE). The inoculum was placed on the center of the agar surface, which enabled visualization of motility across the agar surface.[5] The diameters of the swarming motility zones were measured after incubation at 37°C for 24 hours.

Statistics

The biofilm formation assays were performed in at least four plates and repeated at least three times. The results for each series of experiments were measured as a percentage of the control and are shown as means ± standard deviation. The significance of intergroup differences in the adherent cells was analyzed using student's t test (unpaired t test).

RESULTS

The inhibitory effects of aqueous Tp extracts on bacterial growth and biofilm formation of P. aeruginosa

Aqueous Tp extracts significantly repressed bacterial growth at 0.27% and 0.135% ($P < 0.01$) (Fig. 1). Furthermore, 0.25% and 0.125% aqueous Tp extracts significantly inhibited biofilm formation and bacterial growth ($P < 0.01$) (Fig. 1). However, 0.25% and 0.125% methanolic Tp extracts did not inhibit bacterial growth or biofilm formation (Fig. 1).

The effects of heating and light exposure on the ability of aqueous Tp extracts to inhibit the growth and biofilm formation of P. aeruginosa

The aqueous Tp extracts that were heated at 60°C and 80°C exhibited impaired inhibition of bacterial growth ($P < 0.05$) and biofilm formation ($P < 0.01$) (Fig. 2). However, the extracts exposed to light showed increased inhibitory effects of both bacterial growth ($P < 0.05$) and biofilm formation ($P < 0.05$) compared to the non-exposure extracts (Fig. 3).

The effect of ultrafiltration on the ability of aqueous Tp extracts to inhibit the growth and biofilm formation of P. aeruginosa

The fraction < 10 kDa facilitated bacterial growth ($P < 0.01$), but did not affect biofilm formation (Fig. 4). However, the fraction > 10 kDa did not significantly affect bacterial growth, but it did inhibit biofilm formation ($P < 0.05$) (Fig. 4).

The inhibitory effects of polyphenol-rich Tp extracts on the growth and biofilm formation of P. aeruginosa

The 0.2% polyphenol-rich Tp extracts facilitated bacterial growth ($P < 0.01$) and inhibited biofilm formation ($P < 0.01$) (Fig. 5). However, inhibition of biofilm formation by the polyphenol-rich Tp extracts was significantly decreased compared to the 0.27% aqueous Tp extracts ($P < 0.01$).

The effect of aqueous Tp extracts on the biofilm morphology of P. aeruginosa

We observed quantitative and qualitative differences in the biofilm morphology as well as the extracellular polysaccharide (EPS)-like substances production (Fig. 6). A mature biofilm was noted

in the control; however, only microcolonies were observed in the extract-treated biofilms. We also observed that EPS-like substances were bound to the cells and was also densely coated on the surface of cells in the control. However, production of the EPS-like substances appeared to be decreased in the extract-treated biofilm. No difference in the shape of the cells was observed between the control and extract-treated groups.

		untreated	aqueous Tp extract		methanolic Tp extract	
		control	0.27%	0.135%	0.25%	0.125%
Bacterial growth	Average	100	40.2	44.8	95.4	102.8
	Standard deviation	0	2.8	9.6	7.1	14.0
	P-value		< 0.01	< 0.01	0.29	0.72
Biofilm formation	Average	100	55.8	62.2	108.6	114.8
	Standard deviation	0	6.4	13.7	20.0	20.4
	P-value		< 0.01	< 0.01	0.45	0.24

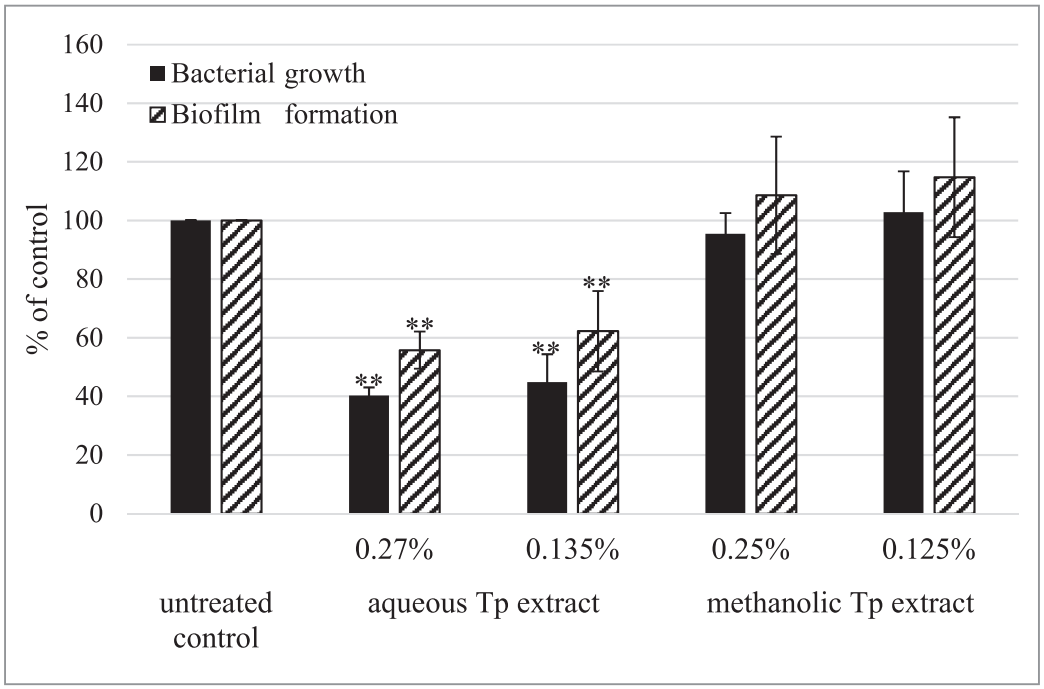

Fig. 1 The inhibitory effects of aqueous Tp extract on PAO1 liquid growth and biofilm formation
0.27% and 0.135% aqueous Tp extracts inhibited both bacterial growth and biofilm formation of PAO1. None of the methanolic Tp extract concentrations displayed any inhibition on bacterial growth or biofilm formation. $^{**}P < 0.01$ versus control.

	untreated control	0.27% aqueous Tp extract		
		unheated	60°C	80°C
Bacterial growth	100	37.3	78.4	92.5
Biofilm formation	100	66.4	106.9	117.6
Standard deviation_Bacterial growth	0	5.4	9.7	9.8
Standard deviation_Biofilm formation	0	12.4	12.6	23.6
P-value_Bacterial growth			< 0.01	< 0.01
P-value_Biofilm formation			0.015	0.018

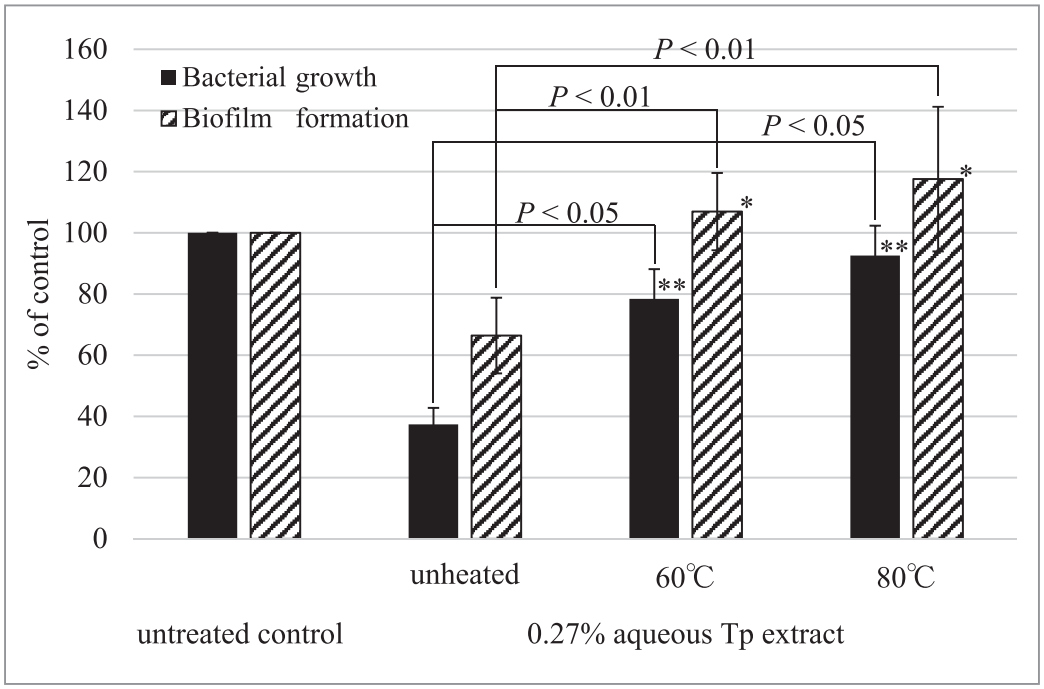

Fig. 2 The effect of heat on the ability of the aqueous Tp extracts to inhibit bacterial growth and biofilm formation

Heat-treatment (60°C or 80°C) significantly impaired the ability of the aqueous Tp extract to inhibit bacterial growth and biofilm formation relative to the untreated control ($P < 0.01$). The unheated extract had a significantly different ($P < 0.05$) effect on both the bacterial growth and biofilm formation from the extracts heating at 60°C and 80°C. $^{**}P < 0.01$ versus control. $^{*}P < 0.05$ versus control.

	untreated control	0.27% aqueous Tp extract	
		non-exposure	light exposure
Bacterial growth	100	40.2	28.4
Biofilm formation	100	55.8	50.4
Standard deviation_Bacterial growth	0	2.8	1.9
Standard deviation_Biofilm formation	0	5.9	3.8
P-value_Bacterial growth		< 0.01	
P-value_Biofilm formation		0.13	

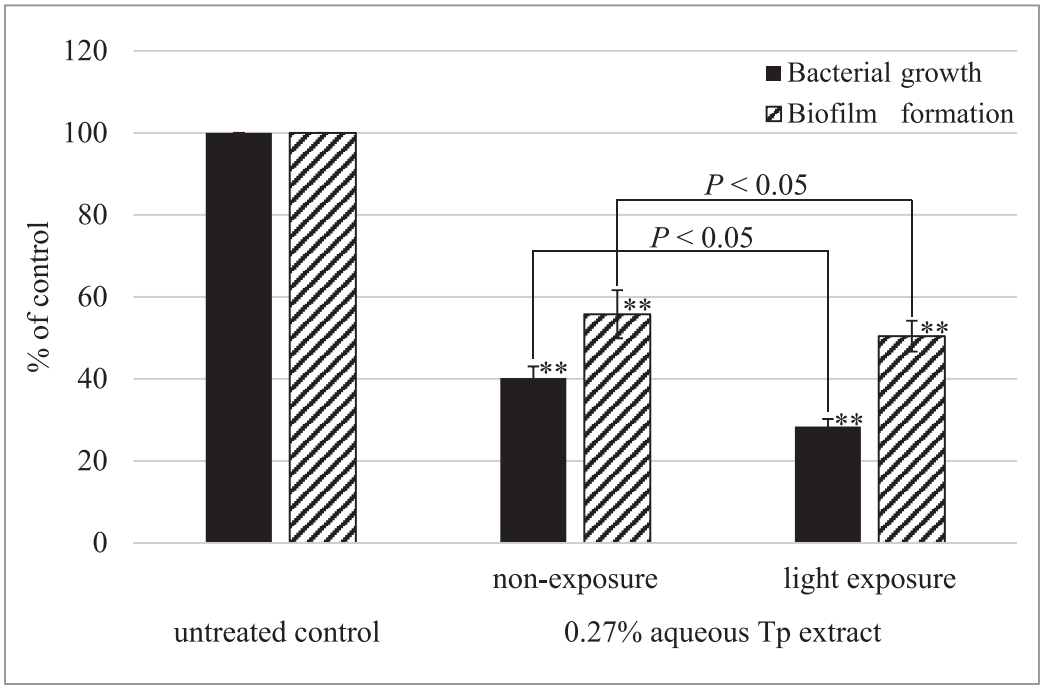

Fig. 3 The effect of light exposure on the ability of the aqueous Tp extracts to inhibit bacterial growth and biofilm formation

The light-exposed aqueous Tp extract inhibited both bacterial growth and biofilm formation. These effects were significantly different from treatment with the extract not exposed to light. $^{**}P < 0.01$ versus control.

	untreated control	0.27% aqueous Tp extract	
		the fraction < 10 kDa	the fraction > 10 kDa
Bacterial growth	100	110.7	103.9
Biofilm formation	100	100.8	88.1
Standard deviation_Bacterial growth	0	6.6	8.5
Standard deviation_Biofilm formation	0	10.6	11.8
P-value_Bacterial growth		< 0.01	0.23
P-value_Biofilm formation		0.84	0.02

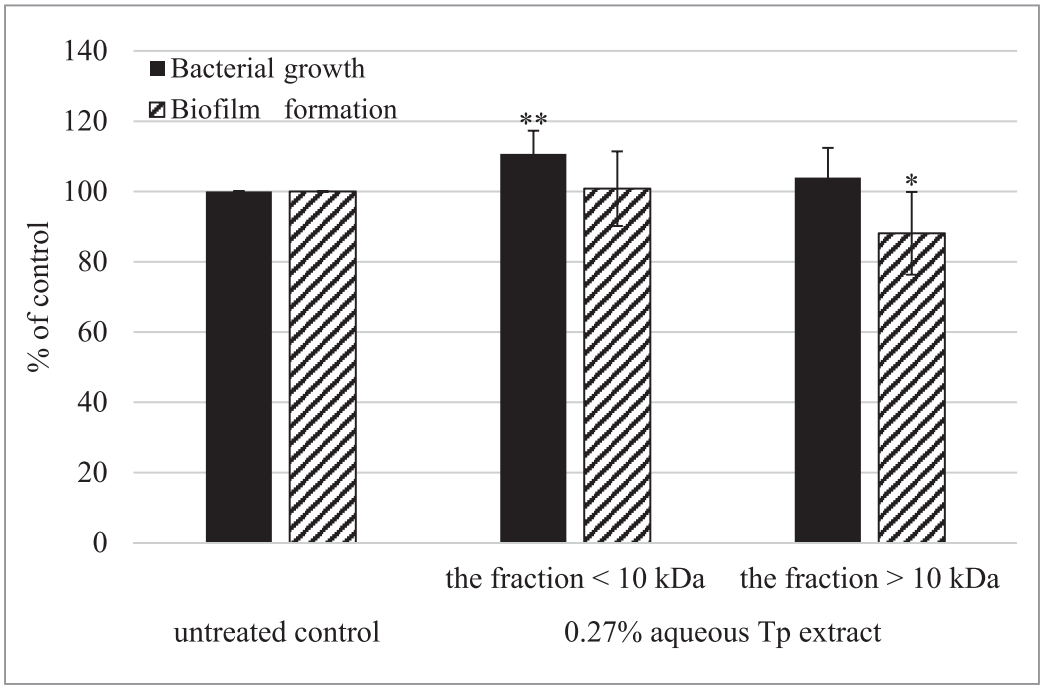

Fig. 4 The effect of ultrafiltration on the ability of the aqueous Tp extract to inhibit bacterial growth and biofilm formation

The < 10 kDa fraction facilitated bacterial growth ($P < 0.01$) and did not affect biofilm formation. The fraction > 10 kDa did not affected bacterial growth, but it did inhibit biofilm formation ($P < 0.05$) compared to the control. $^{**}P < 0.01$ versus control. $^{*}P < 0.05$ versus control.

	untreated control	0.2% polyphenol rich Tp extract	0.27% aqueous Tp extract
Bacterial growth	100	117.3	40.2
Biofilm formation	100	80.9	55.8
Standard deviation_Bacterial growth	0	6.0	2.8
Standard deviation_Biofilm formation	0	7.1	6.4
P-value_Bacterial growth		0.011	< 0.01
P-value_Biofilm formation		0.013	< 0.01
P-value_Bacterial growth_vs. 0.27%		< 0.01	
P-value_Biofilm formation_vs. 0.27%		< 0.01	

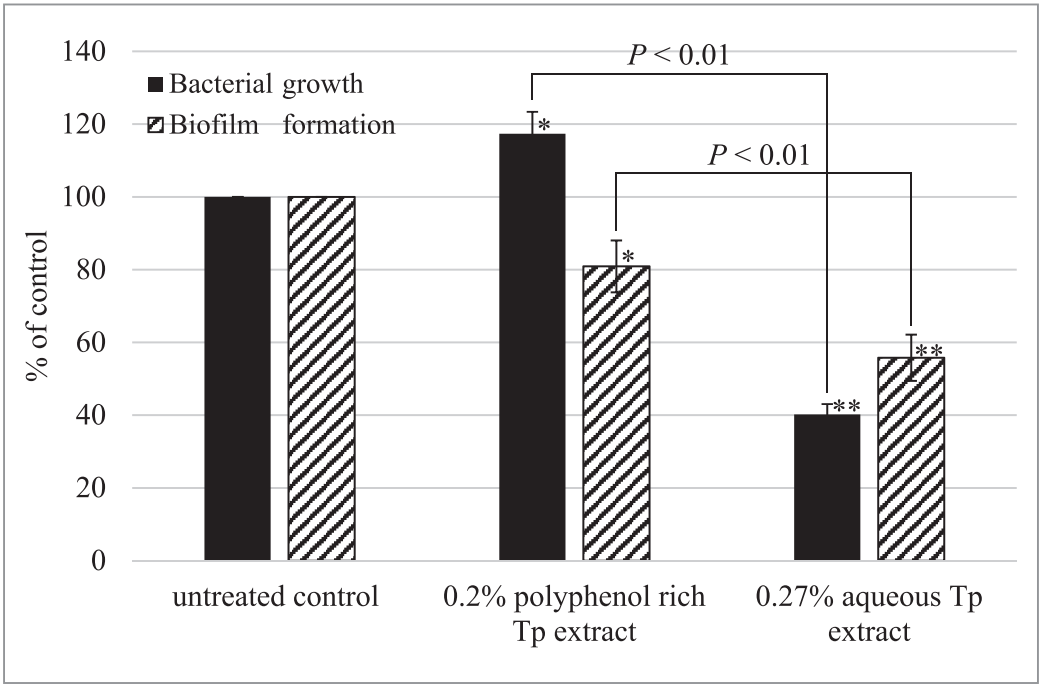

Fig. 5 The inhibitory effect of polyphenol-rich Tp extract on bacterial growth and biofilm formation
The 0.2% polyphenol-rich Tp extract facilitated bacterial growth ($P < 0.01$) and inhibited biofilm formation ($P < 0.01$). However, inhibition of biofilm formation by the polyphenol-rich Tp extract significantly decreased compared to the 0.27% aqueous Tp extract ($P < 0.01$). **$P < 0.01$ versus control. *$P < 0.05$ versus control.

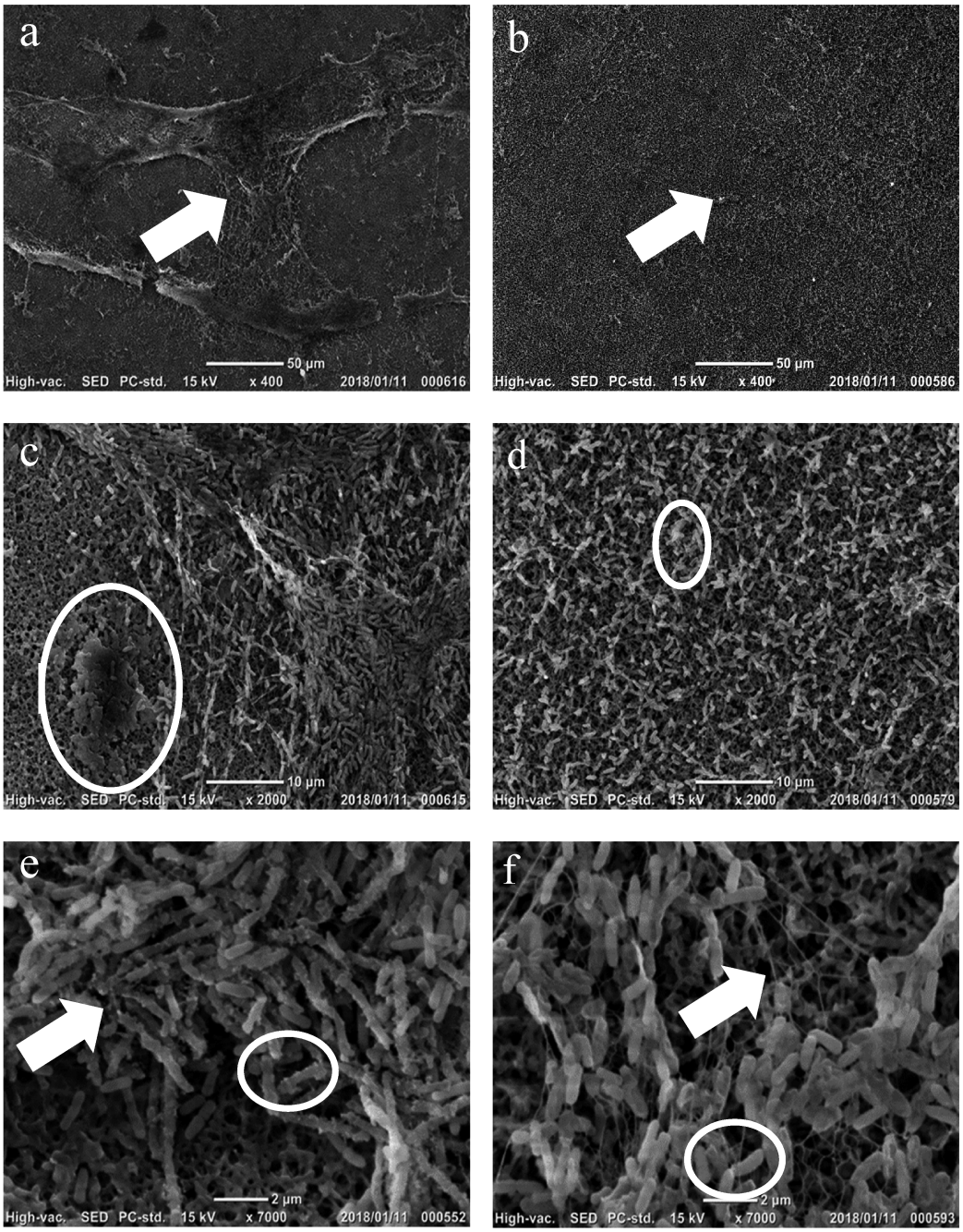

Fig. 6 SEM images of PAO1 biofilms with and without aqueous Tp extract
A mature biofilm formed in the absence of the aqueous Tp extract (a), whereas only microcolonies formed in the presence of the extract (b). Images are ×400 magnification. At a magnification of ×2000, the biofilm without Tp extract did not display a clear distinction of single cells (c). However, the microcolonies that formed in the presence of the Tp extract were clearly distinguished (d). At a magnification of ×7000, the EPS-like substance bound between the cells and the granular EPS-like substance coated the cell surfaces in the absence of the extract (e). The production of these substances decreased in the presence of the extract (f).

Inhibition of swarming motility by aqueous Tp extracts

We were interested in whether the aqueous Tp extract blocked swimming, swarming, and twitching motility involved in the biofilm formation of PAO1. Interestingly, the aqueous Tp extract did not block swimming or twitching (data not shown), but it did block swarming motility (Fig. 7). This finding suggests that aqueous extracts of Tp are able to inhibit biofilm formation via both the inhibition of bacterial growth and the inhibition of swarming motility.

	untreated control	0.27% aqueous Tp extract
Average	19.7	8
Standard deviation	4.7	1.7
P-value vs. control		0.038
% of control		40.7

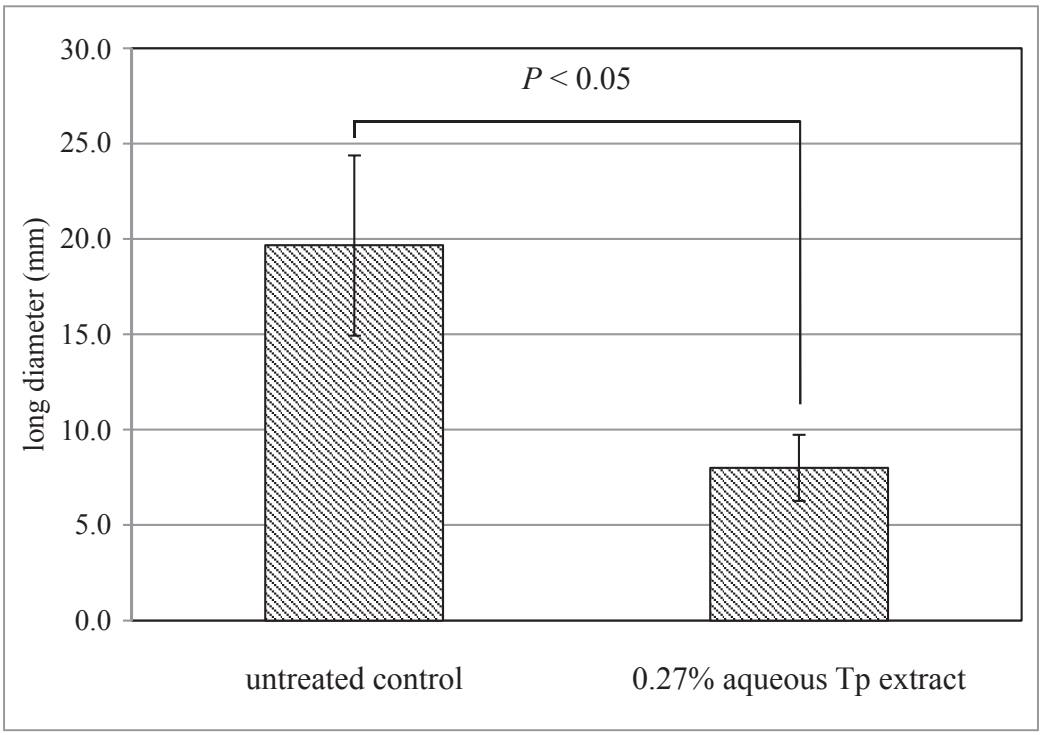

Fig. 7 Inhibition of swarming motility by aqueous Tp extract
Values shown are the mean diameter of the swarming motility zone ± standard deviation, with triplicate plates per experiment. 0.27% aqueous Tp extract significantly inhibited swarming motility (P = 0.038) of PAO1 relative to the untreated control.

DISCUSSION

A biofilm is a mass of bacteria that is capable of evading host defense mechanisms and resisting antibiotics. Yamanaka et al reported the capacity of polyphenols extracted from cranberries to inhibit the *Porphyromonas gingivali*s biofilm formation.[11] Maisuria et al showed that the phenolic-rich maple extract efficiently reduced biofilm formation and repressed multiple-drug resistance genes as well as genes associated with mortality, adhesion, biofilm formation, and virulence.[12]

We hypothesized that Tp extract specifically affects biofilm formation, and we tested our hypothesis on the *P. aeruginosa* strain PAO1. Aqueous Tp extracts significantly inhibited both bacterial growth and biofilm formation; however, methanolic Tp extracts inhibited neither (Fig. 1). These findings suggest the active ingredient is a hydrophilic substance or is decomposed or deactivated by methanol.

The effects of aqueous Tp extracts on the growth inhibition and biofilm inhibition decreased upon heating, indicating that the active substance is hydrolyzed or has a high-order structure (e.g., protein) (Fig. 2). In addition, inhibitory activity increased upon light exposure (Fig. 3). The molecular weight of the active ingredient can be approximated by fractionating the extract into two molecular weight ranges with ultrafiltration. The faction > 10 kDa showed inhibition of biofilm formation, and the faction < 10 kDa did not show inhibition of biofilm formation (Fig. 4). This result indicates that the active compound is a high-molecular weight substance.

Furthermore, we investigated whether the ingredient was a polyphenol. Polyphenol-rich Tp extracts showed inhibition of biofilm formation (Fig. 5), suggesting that the active ingredient is a polyphenol. However, the inhibition of biofilm formation by polyphenol-rich Tp extract was decreased compared with the 0.27% aqueous Tp extract (Fig. 5). We considered that this result indicated unknown substances besides polyphenols affecting bacterial growth and biofilm formation. Junio et al reported that three flavonoids from goldenseal (*Hydrastis canadensis*), sideroxylin, 8-desmethyl-sideroxylin, and 6-desmethyl-sideroxyxylin, synergistically enhanced the antimicrobial activity of the alkaloid berberine, and these flavonoids were missed using traditional bioactivity directed fractionation.[13] Furthermore, Tan et al reported Tp has antibacterial activities against several gram-negative bacteria, *Aeromonas hydrophila* and *Proteus vulgalis* rather than several gram-positive bacteria.[14] Thus, we theorize that the effect of the aqueous Tp extract on bacterial growth and biofilm formation is a result of not only polyphenols, but also potentially multiple other substances that act additively, synergistically, or antagonistically together.

In this study, we quantified biofilm formation and observed biofilm quality. When forming a biofilm, bacteria undergo a switch from reversible attachment to irreversible attachment. Next, adhesins and extracellular polysaccharides are produced, microcolonies are formed, and a biofilm matures as a mushroom-like structure. The large three-dimensional biofilm structure was observed in control cells, while cells treated with the aqueous Tp extracts did not form mature biofilms. Moreover, cell death was not obvious in cells treated with the aqueous Tp extracts, and the mechanism of inhibition of bacterial growth is not distinct. The EPS-like substances were remarkably decreased in Tp-treated cells, as observed by SEM. Moreover, the inhibition of bacterial growth does not necessarily correlate with the inhibition of biofilm formation. Thus, further research using a dynamic biofilm system is needed in order to determine the mechanism by which Tp extracts influence *P. aeruginosa* biofilm formation.

P. aeruginosa can undergo flagellum-mediated swimming motility, in addition to surface-associated swarming and twitching motilities, which are predominantly mediated by hyperflagellation and type-IV, respectively.[4] Swarming has been noted to have an important impact on early biofilm formation.[15] It has been reported that swarming may be a highly organized form of motility involving multiple functions—the regulation of which is remarkably complex.[16] Swarming

motility depends on flagella,[5] c-di-GMP levels,[17] and the production of rhamnolipids.[18] In the present study, aqueous Tp extracts not only limited bacterial surface colonization, but also served as an environmental signal for changing phenotypes. It is considered the signal transduction system regulating biofilm formation undergoes alterations and regulates gene expressions based on several environmental signals. Thus, the exact phase of biofilm formation that is affected by the aqueous extract of Tp remains unknown.

In conclusion, the findings of this study demonstrated that aqueous Tp extract inhibits the liquid growth and biofilm formation of PAO1. This study provides new information on using antimicrobial substances and biofilm inhibitors against *P. aeruginosa*.

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DISCLOSURE STATEMENT

All authors state that there are no conflicts of interests to declare.

REFERENCES

1. Mikkelsen H, Duck Z, Lilley KS, Welch M. Interrelationships between colonies, biofilms, and planktonic cells of Pseudomonas aeruginosa. *J Bacteriol*. 2007;189(6):2411–2416.
2. O'Toole G, Kapla HB, Kolter R. Biofilm formation as microbial development. *Annu Rev Microbiol*. 2000;54(1):49–79.
3. Nadell CD, Xavier JB, Foster KR. The sociobiology of biofilms. *FEMS Microbiol Rev*. 2009; 33(1):206–224.
4. O'Toole GA, Kolter R. Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development. *Mol Microbiol*. 1998;30(2):295–304.
5. O'May C, Tufenkji N. The swarming motility of Pseudomonas aeruginosa is blocked by cranberry proanthocyanidins and other tannin-containing materials. *Appl Environ Microbiol*. 2011;77(9):3061–3067.
6. Inoue T, Shingaki R, Fukui K. Inhibition of swarming motility of Pseudomonas aeruginosa by branched-chain fatty acids. *FEMS Microbiol Lett*. 2008;281(1):81–86.
7. Silva AMAP, Silva AM, Masson R, et al. Avaliação da atividade antimicrobiana da planta Tradescantia pallida Munt (Taboquinha Roxa). *Rev Bras Pl Med*. 2015;17(3):374–378.
8. Konaté K, Hilou A, Mavoungou JF, et al. Antimicrobial activity of polyphenol-rich fractions from Sida alba L. (Malvaceae) against co-trimoxazol-resistant bacteria strains. *Ann Clin Microbiol Antimicrob*. 2012;11(1):5.
9. Matsunaga T, Karim MM. The inhibitory effects of catechins on biofilm formation by the periodontopathogenic bacterium, Eikenella corrodens. *Biosci Biotechnol Biochem*. 2010;74(12):2445–2450.
10. O'Toole GA, Kolter R. Initiation of biofilm formation in Pseudomonas fluorescens WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. *Mol Microbiol*. 1998;28(3):449–461.
11. Yamanaka A, Kouchi T, Kasai K, Kato T, Ishihara K, Okuda K. Inhibitory effect of cranberry polyphenol on biofilm formation and cysteine proteases of Porphyromonas gingivalis. *J Periodontal Res*. 2007;42(6):589–592.
12. Maisuria VB, Hosseinidoust Z, Tufenkji N. Polyphenolic extract from maple syrup potentiates antibiotic susceptibility and reduces biofilm formation of pathogenic bacteria. *Appl. Environ Microbiol*. 2015;81(11):3782–3792.
13. Junio HA, Sy-Cordero AA, Ettefagh KA, et al. Synergy-directed fractionation of botanical medicines: a case study with goldenseal (Hydrastis canadensis). *J Nat Prod*. 2011;74(7):1621–1629.
14. Tan JB, Yap WJ, Tan SY, Lim YY, Lee SM. Antioxidant content, antioxidant activity, and antibacterial activity of five plants from the commelinaceae family. *Antioxidants (Basel)*. 2014;3(4):758–769.
15. Korber DR, Lawrence JR, Sutton B, Caldwell DE. Effect of laminar flow velocity on the kinetics of surface

recolonization by Mot+ and Mot− Pseudomonas fluorescens. *Microb Ecol*. 1989;18(1):1–19.

16. Oura H, Tashiro Y, Toyofuku M, et al. Inhibition of Pseudomonas aeruginosa swarming motility by 1-naphthol and other bicyclic compounds bearing hydroxyl groups. *Appl Environ Microbiol*. 2015;81(8):2808–2818.
17. Caiazza NC, Merritt JH, Brothers KM, O'Toole GA. Inverse regulation of biofilm formation and swarming motility by Pseudomonas aeruginosa PA14. *J Bacteriol*. 2007;189(9):3603–3612.
18. Caiazza NC, Shanks RM, O'Toole GA. Rhamnolipids modulate swarming motility patterns of Pseudomonas aeruginosa. *J Bacteriol*. 2005;187(21):7351–7361.