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Original article

Effects of 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid and its ester derivatives on biofilm formation by two oral pathogens, *Porphyromonas gingivalis* and *Streptococcus mutans*

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Abstract

The aim of this study was to investigate the effect of 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid, active principle isolated from *Acronychia baueri* Schott, and its ester derivatives on biofilm formation by two important oral pathogens, *Porphyromonas gingivalis* and *Streptococcus mutans*. The parent acid and conjugates with vanillic acid, 2-hydroxynaphthoquinone and guaiacol caused a significant and reproducible inhibition of *P. gingivalis* biofilm formation. This effect could be related to the ability of the compounds to inhibit bacterial growth. These compounds also efficiently caused a reduction of biofilm formation by *S. mutans*, a phenomenon not related to growth inhibition. These data suggest that 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid and some of its ester derivatives may have a therapeutic/preventive potential for oral infections.

Keywords: Oral infection; Bacteria; Biofilm; Ferulic acid

1. Introduction

Periodontitis is a destructive inflammatory disorder that leads to the loss of tooth-support. It is initiated by a specific group of Gram-negative anaerobic bacteria, which modulate periodontal tissue destruction through complex interactions with mucosal and immune cells [1,2]. Much evidence points to *Porphyromonas gingivalis* as the key pathogen in chronic periodontitis [1]. The ability of this bacterium to form biofilm and to colonize subgingival sites is a critical step in the initiation of periodontitis. *P. gingivalis* is well known to express several adhesins, associated with either the outer membrane or fimbriae, that promote its adhesion to tooth surfaces, gingival epithelial cells, basement membrane components, erythrocytes and oral bacteria [3]. Dental caries is the direct result of enamel dissolution by acid-producing bacteria inhabiting dental biofilm, especially when the biofilm reaches a critical mass due to poor oral hygiene. *Streptococcus mutans*, known as a major cariogenic bacteria, produces extracellular polysaccharide that contributes to the formation of the dental biofilm [4].

3-(4′-Geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid **1** (Fig. 1) is a secondary metabolite biosynthetically related to ferulic acid in which a geranyl chain is attached to the phenolic group. It has been isolated in 1966 from the bark of *Acronychia baueri* Schott, an Australian small tree belonging to the family of Rutaceae [5]. Although known for four decades, only in the last decade this natural compound showed valuable pharmacological properties that have been recently reviewed [6]. Both **1** and its some structurally simple ester derivatives (e.g. ethyl ester) are considered potential drugs for chemoprevention of different types of cancer, especially those of gastrointestinal tract [6].

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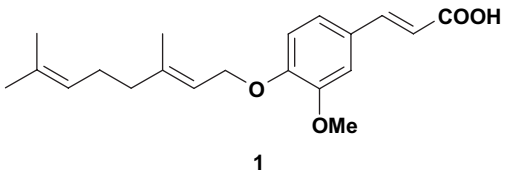


1

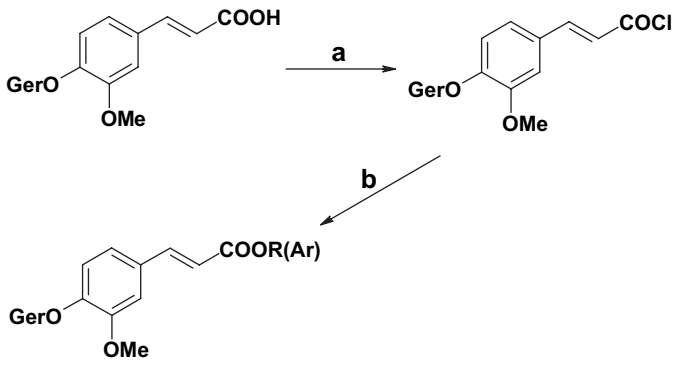
Fig. 1. 3-(4′-Geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid.

In continuation of our research studies aimed to demonstrate the beneficial properties of **1** for human health, we wish to report herein the preparation of some ester derivatives of the parent acid using the following natural compounds possessing an alcohol or phenol moiety: umbelliferone **2** [7], 2-hydroxynaphthoquinone **3** [8], salicylic **4**, ferulic **5**, caffeic **6**, and vanillic **7** acids [9], guaiacol **8** [10], naringenin **9** [11], hydroquinone **10** [12], and anthraflavin **11** [13] which are known to have antimicrobial activity. Thereafter, **1** and its derivatives were tested for their ability to inhibit biofilm formation by two important oral pathogens: *P. gingivalis* and *S. mutans*. It is thought that such a chemical modification may allow synergism of biological action, once the two portions of the ester are cleaved by bacterial lipases.

2. Chemistry

Compound **1** was synthesized in 98% yield as already reported [14]. Esters of **1** were synthesized in two steps by conversion of the acid into the corresponding acyl chloride by reaction with oxalyl chloride in dry diethyl ether at room temperature, followed by its reaction with the alcohol or phenol and triethylamine in dry diethyl ether at room temperature (Scheme 1). Yields of ester derivatives are reported in Table 1. Salicylic, ferulic, caffeic and vanillic acid were conjugated to **1** in form of methyl esters.

Crucial for the success of this synthesis is the time of the reaction of conversion of compound **1** into the corresponding chloride that had to be rigorously of 5 min, while for longer reaction times the acyl chloride went rapidly through decomposition. Moreover each attempt to synthesize esters by direct condensation of the acid with alcohol or phenol in the presence of different reagents like dicyclohexyl carbodiimide or 1,1-carbonyl diimidazole were unsuccessful.



Scheme 1. Reagents and conditions: (a) $(COCl)_2$, dry Et_2O, room temperature for 5 min; (b) ROH (ArOH), Et_3N, dry Et_2O, room temperature for 40 min.

3. Biological results and discussion

The effect of **1**, alcohols **2**–**11** and esters **12**–**21** were first tested for their capacity to inhibit biofilm formation by the periodontopathogenic bacterium *P. gingivalis* (Fig. 2). Compound **1** at 31.3 μg/ml (78.1 μM) caused an inhibition of biofilm formation by *P. gingivalis* of approximately 80%. Among alcohols tested, only 2-hydroxynaphthoquinone **3** and hydroquinone **10** reduced the formation of biofilm by *P. gingivalis*. Even at the lowest concentration tested (3.9 μg/ml; 35.5 μM) **10** caused a reduction (>80%) of biofilm formation. Three ester derivatives of **1** showed a capacity to interfere with biofilm formation by *P. gingivalis*: 2-hydroxynaphthoquinone **13**, vanillic acid **17** and guaiacol **18** derivatives. The inhibition obtained with **13** and **17** was not better than that obtained with compound **1**. However, the guaiacol derivative **18** was much more potent than the parent molecule and was able to significantly ($P \leq 0.05$) inhibit biofilm formation at the lowest concentration tested (3.9 μg/ml; 8.6 μM).

We then investigated whether the effects of the above cited compounds on biofilm formation by *P. gingivalis* were related to an inhibition of bacterial growth. As reported in Table 2, all compounds showed a capacity to reduce growth of *P. gingivalis* that correlates with their ability to inhibit biofilm formation. Preformed biofilms (48 h) of *P. gingivalis* were incubated with the compounds for 4 h prior to determination of cell viability using an XTT assay. None of the compounds caused a significant decrease in viability of *P. gingivalis* suggesting that the antibacterial effect is bacteriostatic rather than bactericidal.

In the second part of the study, the compounds that showed activity on *P. gingivalis* were tested for their effect on biofilm formation by *S. mutans* (Fig. 3). Compound **1** at the lowest concentration tested (3.9 μg/ml; 9.8 μM) still caused a significant and reproducible inhibition ($P \leq 0.05$) of biofilm formation. Compounds **3** and **10** were effective in reducing biofilm formation by *S. mutans* at a concentration down to 31.3 μg/ml (180 and 284.4 μM, respectively). The three derivatives of compound **1** (**13**, **17** and **18**), although they could reduce biofilm formation to some extent, were less effective that the parent molecule. On the contrary to what was observed with *P. gingivalis*, none of the compounds showed a capacity to inhibit growth of *S. mutans* (data not shown). The compounds thus have a specific anti-biofilm effect on *S. mutans*.

To better evaluate the significance of the data obtained, the effect of chlorhexidine, an antimicrobial agent largely used to treat oral infections, on *P. gingivalis* and *S. mutans* was tested. The chlorhexidine minimal inhibitory concentrations on *P. gingivalis* and *S. mutans* were 4 μg/ml (6 μM) and 2 μg/ml (3 μM), respectively. The reduction in biofilm formation observed in the presence of sub-optimal inhibitory concentrations of chlorhexidine correlated with partial growth inhibition of both bacterial species. Therefore, no specific effect on biofilm formation could be associated with chlorhexidine, as previously reported [15].

Colonization and subsequent biofilm formation by oral pathogens such as *P. gingivalis* and *S. mutans* is the initial step in the pathogenesis of oral infections. Biofilms, which

Table 1
Synthesis of ester derivatives of 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid

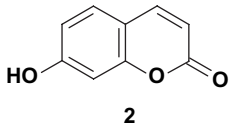
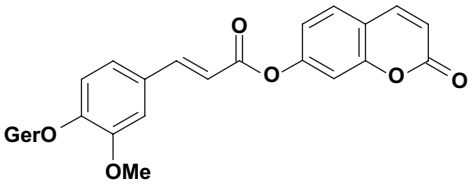
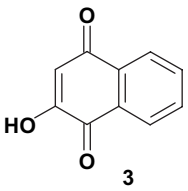
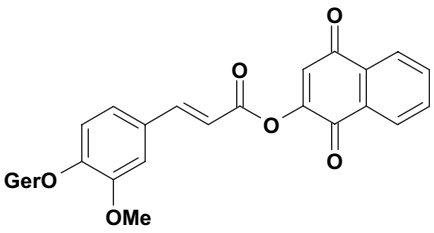
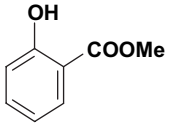
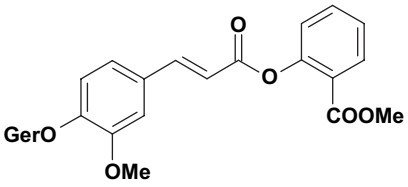
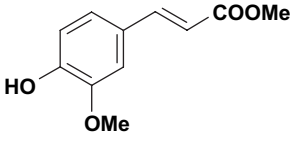
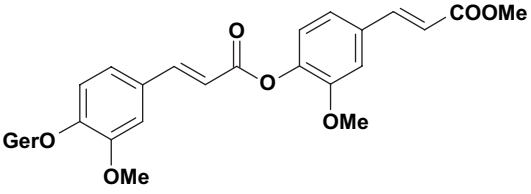
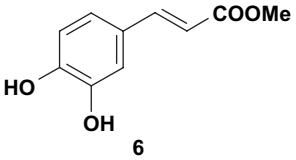
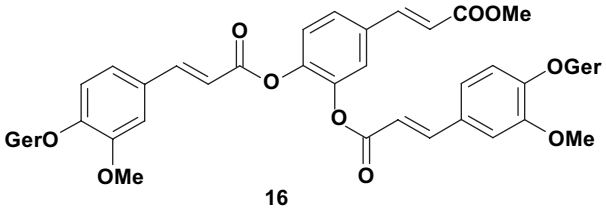
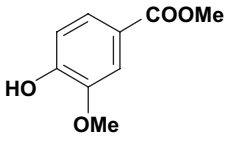
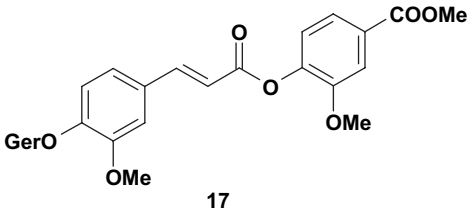
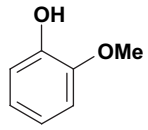
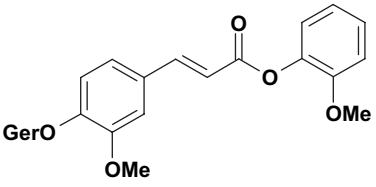
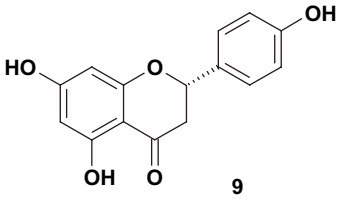
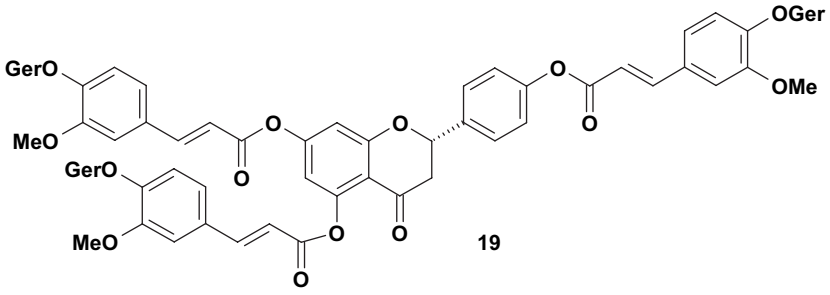
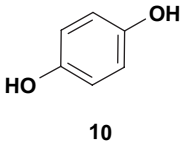
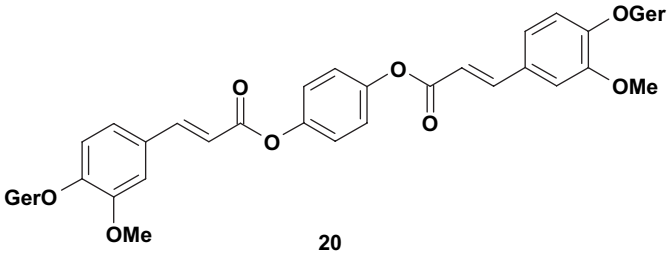
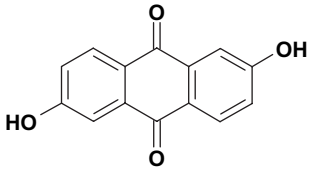
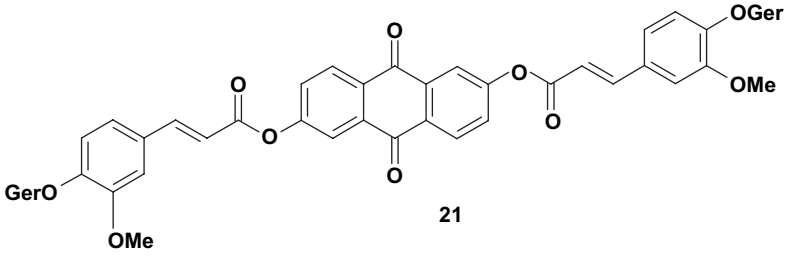
Substrate	Product	Yield (%)[a]
 2	 12	65
 3	 13	95
 4	 14	81
 5	 15	63
 6	 16	80
 7	 17	87

Table 1 (*continued*)

Substrate	Product	Yield (%)[a]
 8	 18	31
 9	 19	69
 10	 20	44
 11	 21	36

[a] Yields of pure isolated product fully characterized by IR, GC–MS, ^{1}H NMR and ^{13}C NMR.

are defined as structured microbial communities attached to surfaces, play an important role in most bacterial infections of the human body. In the oral cavity, biofilms allow bacteria to evade immune defenses and to better resist to mechanical removal and chemotherapeutic agents. Only in the last five years it has been observed that the presence of a terpenyl hydrocarbon chain, either farnesyl, geranyl or isopentenyl, greatly enhance the antibacterial properties of a natural or semisynthetic compounds [16]. In particular natural prenyloxy secondary metabolites of phenylpropanoid biosynthetic origin very recently were shown to exert valuable antimicrobial properties against several bacteria such as *Klebsiella pneumoniae*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, *Salmonella galinnarum*, *Staphylococcus aureus* and *Helicobacter pylori* [17–19]. Among this latter class of natural products 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid **1** was seen to be pharmacologically active against several biological targets [6,14,20], while other structurally similar molecules (e.g. the acid lacking the methoxy group in position 3) do not share the same pharmacological properties. This allowed us to exclude a non-specific effects on the lipid matrix of cell membranes, acting like a surfactant, in considering the biological action of compound **1**. Although the underlying mechanism is not known at the moment, although it is currently under investigation, the same hypothesis could be made in explaining the observed effect on prevention of biofilm formation by 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid. It is noteworthy that, although

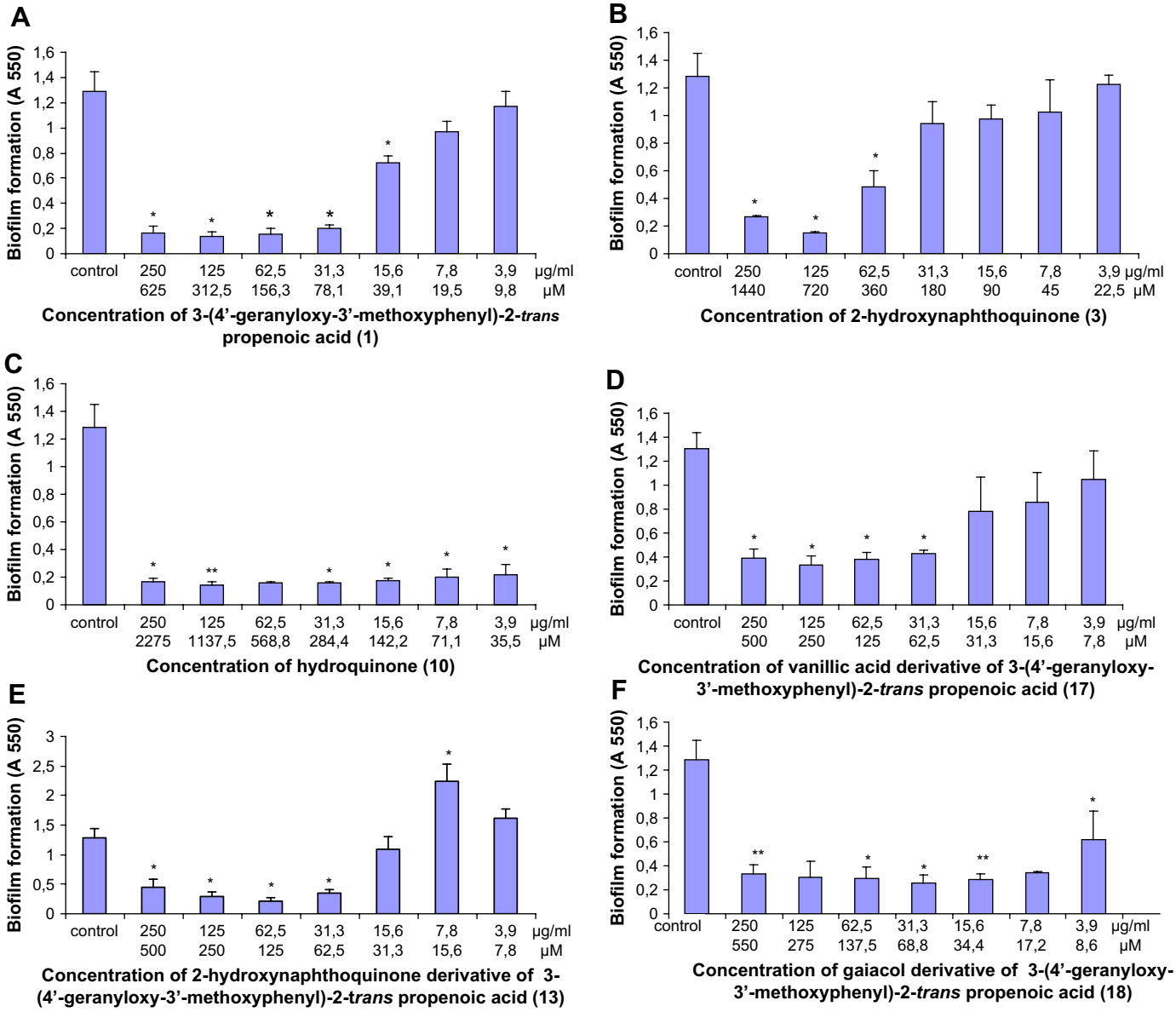


Fig. 2. Effect of **1** (A), **3** (B), **10** (C), **17** (D), **13** (E), and **18** (F) on biofilm formation by *P. gingivalis*.

esterification of acid **1** with other phenolic natural compounds greatly improve the lipophilicity of the resulting adduct, leading in theory to a better capacity to permeate biofilm surface and thus to a better antimicrobial activity, only in one case the activity of the ester was superior to that of the parent acid. Moreover, compound **1** in many cases performed better than other parent natural products. The preliminary data about the capacity of compound **1** to inhibit biofilm formation herein described will prompt us to use 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid as a lead compound to draw and synthesize properly structured isosters (e.g. those deriving from substation of the methoxy group in position 3 with an halogen atom) in order to improve the title pharmacological profile. In conclusion, overall, our data suggest that 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid and some of its ester derivatives (vanillic acid, 2-hydroxynaphthoquinone and guaiacol) may have a therapeutic potential for pharmacological treatment of oral infections by reducing the capacity of *P. gingivalis* and *S. mutans* to form biofilm.

4. Experimental protocols

4.1. Synthesis of esters of 3-(4′-geranyloxy-3′-methoxyphenyl)-2-trans propenoic acid — general procedure

To a solution of 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid **1** (0.55 mmol) in anhydrous Et_2O (2.5 ml), oxalyl chloride (1.11 mmol) was added and the resulting solution was stirred under N_2 for 5 min at room

Table 2
Effect of 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid, 2-hydroxynaphthoquinone, hydroquinone, vanillic acid derivative of 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid, 2-hydroxynaphthoquinone derivative of 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid, and guaiacol derivative of 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid on growth of *P. gingivalis*

Compound	*P. gingivalis* relative growth (%)							
	Control	250[a]	125[a]	62.5[a]	31.2[a]	15.6[a]	7.8[a]	3.9[a]
1	100 ± 12	30 ± 8*	13 ± 5*	9 ± 5*	15 ± 6*	34 ± 12*	68 ± 23	81 ± 26
3	100 ± 12	27 ± 9*	49 ± 15*	79 ± 16	86 ± 14	92 ± 15	100 ± 14	103 ± 10
10	100 ± 12	15 ± 11*	14 ± 9*	13 ± 12*	17 ± 6*	15 ± 5*	27 ± 15*	45 ± 8*
13	100 ± 12	21 ± 11*	17 ± 7*	16 ± 3*	34 ± 10*	105 ± 15	124 ± 4	110 ± 17
17	100 ± 12	39 ± 7*	40 ± 11*	49 ± 14*	72 ± 16	76 ± 18	87 ± 18	98 ± 14
18	100 ± 12	39 ± 6*	32 ± 10*	25 ± 14*	28 ± 15*	22 ± 10*	25 ± 11*	61 ± 9*

*$P < 0.05$ in Student's *t*-test.
[a] Dose expressed in μg/ml.

temperature. The solvent was evaporated under vacuum, the resulting syrup was dissolved in anhydrous Et_2O (3 ml) and to this solution was added dropwise over a period of 30 min a solution of phenol derivative (0.48 mmol) and Et_3N (1.2 mmol) in anhydrous Et_2O (3 ml). The resulting mixture was stirred for 10 min and the white precipitate formed was filtered under vacuum and washed twice with Et_2O (5 ml). The filtrate was then extracted twice with a 1% solution of citric acid (10 ml), the organic phase washed twice with a 1% solution of $NaHCO_3$ (5 ml), dried over anhydrous Na_2SO_4 and the solvent evaporated under vacuum to yield the desired ester.

*4.1.1. Umbelliferyl (2E)-3-(4-{[(2E)-2,7-dimethylocta-2,6-dienyl]oxy}-3-methoxyphenyl)prop-2-enoate (**12**)*

White solid; yield 65%; mp: 186–188 °C; IR (KBr): 1687, 1684 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ): 1.62 (s, 3H), 1.69 (s, 3H), 1.75 (s, 3H), 2.09–2.20 (m, 4H), 3.95 (s, 3H), 4.69–4.72 (m, 2H), 4.97–5.13 (m, 2H), 5.47–5.56 (m, 1H), 6.48 (d, 1H, $J = 9.9$ Hz), 6.74 (d, 1H, $J = 12.4$ Hz), 6.92 (d, 1H, $J = 8.1$ Hz) 7.15–7.29 (m, 4H), 7.54 (d, 1H, $J = 8.1$ Hz), 7.73 (d, 1H, $J = 9.9$ Hz), 7.86 (d, 1H, $J = 12.4$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$ δ) 16.5, 17.6, 25.4, 26.0, 39.4, 55.9, 65.9, 110.1, 111.6, 114.3, 115.3, 115.9, 116.7, 118.0, 119.4, 121.9, 123.6, 128.4, 130.1, 131.8, 141.6, 144.1, 144.4, 146.2, 150.7, 156.1, 158.0, 161.7, 165.3. Anal. Calcd for $C_{29}H_{30}O_6$: C, 73.40; H, 6.37; O, 20.23. Found: C, 73.39; H, 6.34; O, 20.24.

*4.1.2. 1,4-Dioxo-1,4-dihydronaphthalen-2-yl (2E)-3-(4-{[(2E)-2,7-dimethylocta-2,6-dienyl]oxy}-3-methoxyphenyl)prop-2-enoate (**13**)*

Yellowish solid; yield 95%; mp: 208–209 °C; IR (KBr): 1705, 1695, 1685 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ): 1.62 (s, 3H), 1.69 (s, 3H), 1.73 (s, 3H), 2.08–2.21 (m, 4H), 3.97 (s, 3H), 4.71–4.73 (m, 2H), 4.98–5.11 (m, 2H), 5.51–5.59 (m, 1H), 6.67 (d, 1H, $J = 12.3$ Hz) 6.94–8.17 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$ δ) 16.2, 17.4, 25.1, 25.6, 26.2, 39.5, 55.9, 65.9, 107.5, 111.6, 114.4, 119.8, 121.9, 123.9, 127.1, 127.3, 129.6, 131.2, 131.3, 132.8, 133.5, 134.1, 134.9, 141.6, 144.3, 147.4, 146.9, 150.7, 152.2, 161.8, 176.7, 183.8. Anal. Calcd for $C_{30}H_{30}O_6$: C, 74.06; H, 6.21; O, 19.73. Found: C, 74.03; H, 6.19; O, 19.70.

*4.1.3. Methyl 2-{[(2E)-3-(4-{[(2E)-2,7-dimethylocta-2,6-dienyl]oxy}-3-methoxyphenyl)prop-2-enoyl]oxy}benzoate (**14**)*

Yellow solid; yield 81%; mp: 182–183 °C; IR (KBr): 1698, 1695 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ): 1.67 (s, 3H), 1.69 (s, 3H), 1.75 (s, 3H), 2.10–2.18 (m, 4H), 3.98 (s, 3H), 3.99 (s, 3H), 4.68–4.72 (m, 2H), 5.06–5.09 (m, 1H), 5.50–5.53 (m, 1H), 6.54–8.08 (m, 9H); ^{13}C NMR (50 MHz, $CDCl_3$ δ) 16.4, 17.7, 25.7, 26.6, 27.5, 39.6, 51.4, 56.1, 67.9, 111.7, 114.2, 116.7, 119.4, 121.0, 121.9, 123.3, 123.9, 127.8, 128.6, 131.9, 132.1, 133.2, 135.2, 144.8, 146.2, 150.9, 155.0, 168.2, 168.5. Anal. Calcd for $C_{28}H_{32}O_6$: C, 72.39; H, 6.94; O, 20.66. Found: C, 72.40; H, 6.93; O, 20.68.

*4.1.4. 2-Methoxy-4-[(1E)-3-methoxy-3-oxoprop-1-enyl]phenyl (2E)-3-(4-{[(2E)-2,7-dimethylocta-2,6-dienyl]oxy}-3-methoxyphenyl)prop-2-enoate (**15**)*

Yellowish solid; yield 63%; mp: 159–161 °C; IR (KBr): 1695, 1692 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ): 1.62 (s, 3H), 1.69 (s, 3H), 1.75 (s, 3H), 2.11–2.20 (m, 4H), 3.92 (s, 3H), 3.94 (s, 3H), 3.97 (s, 3H), 4.68–4.71 (m, 2H), 5.09–5.11 (m, 1H), 5.49–5.53 (m, 1H), 6.51–7.89 (m, 7H); ^{13}C NMR (50 MHz, $CDCl_3$ δ) 16.2, 17.6, 25.8, 26.9, 27.2, 39.4, 55.7, 55.9, 67.9, 111.7, 112.9, 114.0, 117.7, 118.4, 121.5, 123.6, 123.8, 123.9, 127.8, 128.5, 131.5, 132.1, 132.6, 139.2, 144.2, 144.7, 146.4, 150.5, 152.9, 164.9, 169.8. Anal. Calcd for $C_{31}H_{36}O_7$: C, 71.52; H, 6.97; O, 21.51. Found: C, 71.50; H, 6.98; O, 21.50.

*4.1.5. Methyl 4-{[(2E)-3-(4-{[(2E)-2,7-dimethylocta-2,6-dienyl]oxy}-3-methoxyphenyl)prop-2-enoyl]oxy}-3-methoxybenzoate (**16**)*

Yellowish solid; yield 80%; mp: 162–163 °C; IR (KBr): 1698, 1695 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ): 1.62 (s, 3H), 1.69 (s, 3H), 1.74 (s, 3H), 2.11–2.19 (m, 4H), 3.94 (s, 3H), 3.95 (s, 3H), 3.97 (s, 3H), 4.68–4.71 (m, 2H), 5.06–5.11 (m, 1H), 5.49–5.53 (m, 1H), 6.51–7.89 (m, 8H); ^{13}C NMR (50 MHz, $CDCl_3$ δ) 16.5, 17.6, 25.6, 26.9, 27.3, 39.6, 51.2, 55.9, 56.0, 67.9, 111.7, 112.8, 114.0, 117.7, 121.9, 122.3, 123.6, 126.0, 127.7, 128.8, 131.5, 132.1, 144.8, 146.0, 146.2, 149.2, 150.4, 164.8, 166.2. Anal. Calcd for

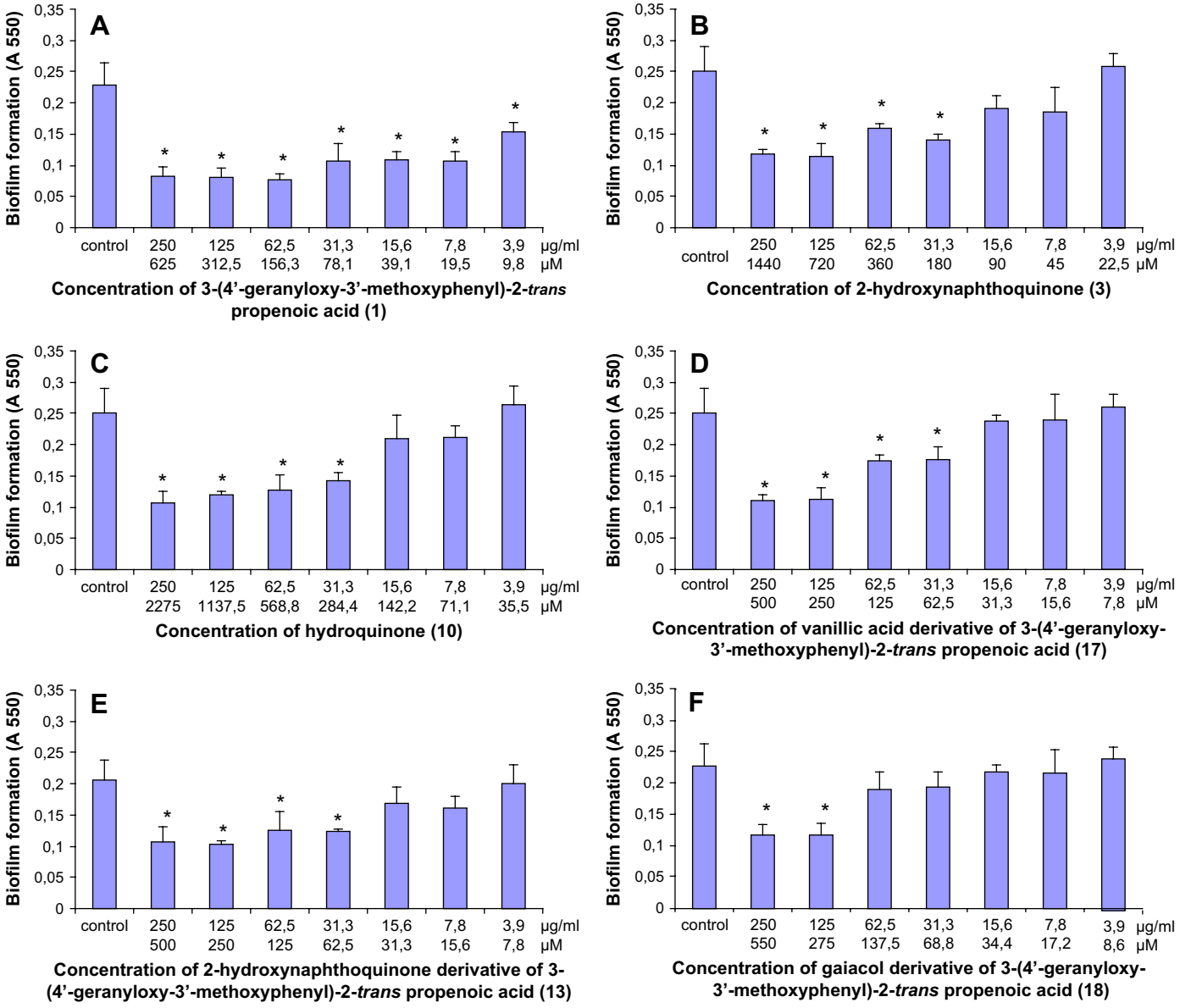


Fig. 3. Effect of **1** (A), **3** (B), **10** (C), **17** (D), **13** (E), and **18** (F) on biofilm formation by *S. mutans*.

$C_{29}H_{34}O_7$: C, 70.43; H, 6.93; O, 22.64. Found: C, 70.41; H, 6.92; O, 22.65.

*4.1.6. 2-Methoxyphenyl (2E)-3-(4-{[(2E)-3,7-dimethylocta-2,6-dienyl]oxy}-3-methoxyphenyl)prop-2-enoate (**17**)*

Orange solid; yield 87%; mp: 145–147 °C; IR (KBr): 1696 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ): 1.62 (s, 3H), 1.69 (s, 3H), 1.72 (s, 3H), 2.11–2.20 (m, 4H), 3.91 (s, 3H), 3.94 (s, 3H), 4.68–4.71 (m, 2H), 5.07–5.12 (m, 1H), 5.50–5.53 (m, 1H), 6.53–7.86 (m, 9H); ^{13}C NMR (50 MHz, $CDCl_3$ δ) 16.2, 17.5, 25.4, 26.2, 39.4, 55.8, 55.9, 65.9, 111.6, 114.4, 114.8, 117.7, 119.9, 121.9, 123.8, 123.9, 125.0, 126.6, 128.8, 131.3, 141.7, 142.5, 144.4, 146.2, 150.8, 151.2, 165.0. Anal. Calcd for $C_{27}H_{32}O_5$: C, 74.29; H, 7.39; O, 18.33. Found: C, 74.28; H, 7.37; O, 18.35.

*4.1.7. 4-(5,7-Bis{[(2E)-3-(4-{[(2E)-3,7-dimethylocta-2,6-dienyl]oxy}-3-methoxyphenyl)prop-2-enoyl]oxy}-4-oxo-3,4-dihydro-2H-chromen-2-yl)phenyl (2E)-3-(4-{[(2E)-3,7-dimethylocta-2,6-dienyl]oxy}-3-methoxyphenyl)prop-2-enoate (**18**)*

Yellowish solid; yield 31%; mp: 262–264 °C (d); IR (KBr): 1695, 1693 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ): 1.64 (s, 9H), 1.68 (s, 9H), 1.75 (s, 9H), 2.09–2.26 (m, 14H), 3.95 (s, 9H), 4.69–4.75 (m, 6H), 5.08–5.56 (m, 7H), 6.53–7.82 (m, 20H); ^{13}C NMR (50 MHz, $CDCl_3$ δ) 16.3, 17.5, 25.6, 26.4, 39.3, 55.6, 65.9, 78.5, 106.1 108.3, 111.7, 113.1, 114.5, 116.6, 116.7, 119.8, 121.6, 121.8, 123.9, 128.7, 130.0, 131.3, 135.9, 142.0, 144.4, 146.2, 150.1, 150.8, 153.5, 158.4, 165.2, 165.7, 169.3, 189.8. Anal. Calcd for $C_{75}H_{84}O_{14}$: C, 74.48; H, 7.00; O, 18.52. Found: C, 74.49; H, 7.02; O, 18.51.

*4.1.8. 4-{[(2E)-3-(4-{[(2E)-3,7-Dimethylocta-2,6-dienyl]oxy}-3-methoxyphenyl)prop-2-enoyl]oxy}phenyl (2E)-3-(4-{[(2E)-3,7-dimethylocta-2,6-dienyl]oxy}-3-ethylphenyl)prop-2-enoate (**19**)*

Bright yellow solid; yield 69%; mp: 232–233 °C (d); IR (KBr): 1693 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ): 1.61 (s, 6H), 1.67 (s, 6H), 1.70 (s, 6H), 2.09–2.17 (m, 8H), 3.93 (s, 6H), 4.67–4.72 (m, 4H), 5.08–5.12 (m, 4H), 5.52–5.56 (m, 2H), 6.53–7.67 (m, 14H); ^{13}C NMR (50 MHz, $CDCl_3$ δ) 16.1, 17.4, 25.7, 26.3, 39.4, 55.8, 65.7, 111.8, 114.3, 116.8, 119.7, 119.8, 121.8, 123.8, 128.5, 131.5, 141.4, 144.2, 145.4, 146.2, 150.9, 165.8. Anal. Calcd for $C_{46}H_{54}O_9$: C, 77.02; H, 7.70; O, 15.28. Found: C, 77.01; H, 7.68; O, 15.30.

*4.1.9. 6-{[(2E)-3-(4-{[(2E)-2,7-Dimethylocta-2,6-dienyl]oxy}-3-methoxyphenyl)-1-methyleneprop-2-enyl]oxy}-9,10-dimethylene-9,10-dihydroanthracen-2-yl (2E)-3-(4-{[(2E)-2,7-dimethylocta-2,6-dienyl]oxy}-3-methoxyphenyl)prop-2-enoate (**20**)*

Yellowish solid; yield 44%; mp: 252–255 °C (d); IR (KBr): 1695 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ): 1.63 (s, 6H), 1.67 (s, 6H), 1.70 (s, 6H), 2.07–2.20 (m, 8H), 3.97 (s, 6H), 4.67–4.71 (m, 4H), 5.08–5.11 (m, 2H), 5.51–5.54 (m, 2H), 6.50–8.43 (m, 16H); ^{13}C NMR (50 MHz, $CDCl_3$ δ) 16.2, 17.6, 25.6, 26.1, 39.4, 55.9, 65.8, 111.6, 114.7, 116.7, 119.8, 120.3, 121.9, 123.8, 127.6, 128.4, 129.2, 131.3, 132.0, 137.6, 141.8, 144.4, 146.9, 150.7, 157.0, 165.7, 182.9. Anal. Calcd for $C_{54}H_{56}O_{10}$: C, 74.98; H, 6.53; O, 18.50. Found: C, 74.95; H, 6.51; O, 18.46.

*4.1.10. Methyl (2E)-3-(3,4-bis{[(2E)-3-(4-{[(2E)-3,7-dimethylocta-2,6-dienyl]oxy}-3-methoxyphenyl)prop-2-enoyl]oxy}phenyl)prop-2-enoate (**21**)*

Yellowish solid; yield 36%; mp: 208–210 °C; IR (KBr): 1699, 1695 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ): 1.64 (s, 6H), 1.68 (s, 6H), 1.72 (s, 6H), 2.11–2.20 (m, 8H), 3.97 (s, 6H), 3.99 (s, 3H), 4.65–4.72 (m, 4H), 5.05–5.11 (m, 2H), 5.50–5.56 (m, 2H), 6.40–7.82 (m, 16H); ^{13}C NMR (50 MHz, $CDCl_3$ δ) 16.5, 17.9, 25.4, 26.0, 39.3, 51.4, 55.9, 65.9, 111.6, 114.3, 116.7, 117.8, 119.4, 121.9, 122.0, 123.7, 126.6, 128.5, 129.5, 131.8, 134.8, 136.5, 141.3, 144.4, 144.6, 146.1, 146.4, 150.7, 164.8, 164.9, 168.2. Anal. Calcd for $C_{50}H_{58}O_{10}$: C, 73.33; H, 7.14; O, 19.54. Found: C, 73.35; H, 7.11; O, 19.56.

4.2. Bacteria and culture conditions

P. gingivalis ATCC 33277 and *S. mutans* ATCC 25175 were used throughout the study. Bacteria were routinely grown in Todd Hewitt Broth (BBL Microbiology Systems, Cockeysville, MD) supplemented with hemin (10 μg/ml) and vitamin K (1 μg/ml) (THB-HK) and incubated for 24 h in an anaerobic chamber ($N_2/H_2/CO_2$ 75/10/15) at 37 °C.

4.3. Effect on growth and biofilm formation

The effect of 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid **1**, alcohols **2**–**11** and ester derivatives **12**–**21** on growth and biofilm formation by *P. gingivalis* was determined in a microplate. A 24 h culture of *P. gingivalis* in THB-HK was diluted in fresh broth medium to obtain an optical density at 660 nm (OD_{660}) of 0.07. Samples (100 μl) were added to the wells of a 96-well tissue culture plate (Sarstedt, Newton, NC) containing 100 μl of serial dilutions (500–7.8 μg/ml) of sterile compounds in THB-HK. Control wells with no compounds were also inoculated. After incubation for 48 h at 37 °C under anaerobic conditions, the absorbance at 660 nm (A_{660}) was recorded. Thereafter, spent media and free-floating bacteria were removed by aspiration using a 26 G needle and the wells were gently washed three times with distilled water. The bacterial biofilms were stained with 0.04% crystal violet (100 μl) for 15 min. The wells were washed four times with distilled water to remove unbound crystal violet dye and dried for 2 h at 37 °C. After adding 100 μl of 95% (v/v) ethanol to each well, the plate was shaken for 10 min to release the stain from the biofilms and the absorbance at 550 nm (A_{550}) was recorded. A preliminary assay revealed that the compounds tested, even at the highest concentration, did not affect the reading of the A_{550} values. Assays were run in triplicate and the means ± standard deviations of two independent experiments were calculated. Compounds that showed an inhibitory effect on biofilm formation by *P. gingivalis* were then tested for their capacity to interfere with biofilm formation by *S. mutans*. Lastly, to compare the effect of 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid **1**, alcohols **2**–**11** and ester derivatives **12**–**21** with a known antimicrobial agent used in dentistry, chlorhexidine was tested.

4.4. Effect on bacterial viability

Compounds showing a capacity to inhibit biofilm formation by *P. gingivalis* were selected and tested for their effect on bacterial viability using the tetrazolium sodium 3′-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}-bis (4-methoxy-6-nitro)-benzene sulfonic acid hydrate (XTT; Sigma–Aldrich Canada Ltd., Oakville, Ontario) reduction assay. Briefly, XTT was dissolved in PBS at 1 mg/ml and menadione was prepared in acetone at 1 mM. The XTT/menadione reagent was prepared fresh and contained 12.5 parts XTT/1 part menadione. Bacterial biofilms were prepared as above and treated with compounds (250–3.9 μg/ml) for 4 h (anaerobiosis, 37 °C) prior to adding 25 μl XTT/menadione. After 1 h at 37 °C, the absorbance at 490 nm (A_{490}) was read using a microplate reader.

4.5. Statistical analyses

Differences between means were analyzed for statistical significance using Student's *t*-test. Differences were considered significant at the 0.05 level (*P* value).

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