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Article

Synergistic Antimicrobial and Antibiofilm Activity of Panax Ginseng, Symphytum Officinale, and Metronidazole against *P. gingivalis*

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Abstract: *Background*: The biofilm-forming bacteria *Porphyromonas gingivalis* (*P. gingivalis*) is primarily responsible for periodontal disorders, which continue to be a global health issue. Despite the widespread use of antibiotics like metronidazole, other potent therapeutic a crucial quorum sensing molecule implicated in the formation of *P. gingivalis* biofilm. Approaches are now being explored due to the growing challenge of antibiotic resistance. *Objective*: In particular, chemicals from Symphytum Officinale (S) and Panax Ginseng (G) plants will be examined in this study to assess if they have the ability to inhibit biofilm and to prevent the development of acylated homoserine lactones (AHLs), a quorum sensing molecule. *Materials and Methods*: With Metronidazole serving as a control, the antibacterial effects of these substances (Symphytum Officinale and Panax Ginseng) were examined against a standard strain and a clinical isolate of *P. gingivalis*. Dilutions of these plant extracts were used either alone (G+S) or in conjunction with metronidazole (G+S+F), to assess their antibacterial activity using antibacterial susceptibility test (inhibition zone), biofilm inhibition and disruption assay (optical density) and quorum sensing inhibition assay (AHL). *Results:* The combinations of Symphytum officinale, Panax Ginseng and metronidazole (S+G+F) showed the maximum effectiveness with highest zone of inhibition (25.000±0.001 mm) and biofilm inhibition (98.46%), and were comparable to G+S (inhibition zone of 24.341±0.593 mm and biofilm inhibition of 97.76%), and significantly different in the degree of biofilm inhibition with different treatment scenarios. Additionally, the plant's extracts and combinations are specific concentrations had a significant effect on the suppression of the generation of AHL ($p < 0.05$). *Conclusion:* According to preliminary research, these plant-derived substances, especially when paired with metronidazole, significantly inhibited the growth of *P. gingivalis* biofilm providing valuable information for the development of innovative therapeutic approaches for periodontitis and other biofilm-associated illnesses.

Keywords: periodontal diseases; *Porphyromonas gingivalis*; plant extract; Symphytum Officinale; Panax Ginseng; metronidazole; acylated homoserine lactones; biofilm; quorum sensing

1. Introduction

Periodontal conditions impact a sizeable section of the world's population and, if ignored, can lead to tooth loss [1]. The gram-negative, anaerobic bacterium *P. gingivalis* is usually considered as one of the pathogens connected to the periodontal problems because of its ability to form biofilms on oral tissues [2]. An organized bacterial colony that is protected by a self-produced polymeric matrix and a biofilm has an advantage over the host's defenses and conventional antibiotic therapies [3].

The bacterial cell to cell communication mechanism known as quorum sensing (QS) is essential for the development of biofilms and other virulence traits [4]. In Gram-negative bacteria like *P. gingivalis*, acylated homoserine lactones (AHLs) play a crucial signaling function in the quorum sensing process [5]. In order to treat chronic disorders like periodontitis, approaches to stop AHL production may lower the activity of bacteria and prevent the formation of biofilms. [6].

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Metronidazole, a nitroimidazole antibiotic, is an excellent treatment option for bacterial infections caused by *P. gingivalis*, are also considered .It functions by damaging bacterial DNA and obstructing protein synthesis [7]. However, the rise in antibiotic resistance threatens the antibiotic's long-term usefulness, necessitating the search for alternative therapies [8].

In this respect, compounds made from plants have attracted a lot of interest because of their shown efficacy against several bacterial infections. Comfrey (Symphytum Officinale) and Panax Ginseng have shown significant antibacterial properties in earlier studies [9]. Comfrey has a number of bioactive compounds with anti-inflammatory, antibacterial, and wound-healing activities, including allantoin, rosmarinic acid, and mucilage [10]. On the other hand, Panax Ginseng containing ginsenosides and saponins, a herb used in East Asian traditional medicine, has been found to have strong antibacterial and anti-inflammatory properties [11]. Ginsenosides are known for having potent antibacterial and anti-inflammatory properties [13]. The examination of these organic compounds for their capacity to inhibit quorum sensing and biofilm growth may pave the way for the development of innovative approaches to treating periodontal disease [12, 14].

Given the growing interest in phytochemicals as potential therapeutic agents, the findings of this study may help in the development of more effective and long-lasting techniques for treating periodontal problems [18]. Given the threat that antibiotic resistance poses to the global health, it is more crucial than ever to find novel plant-based antibacterial medicines [19]. Studying how these two herbs interact with *P. gingivalis* may thus indicate the potential therapeutic value of Symphytum Officinale and Panax Ginseng in the management and treatment of periodontal disease [20].

However, little is known about the specific ways that Panax ginseng and Symphytum Officinale interact with *P. gingivalis*, particularly in relation to the inhibition of biofilm development and the production of AHLs [14]. This is despite the fact that both Panax ginseng and Symphytum Officinale have well-established antibacterial abilities [15]. Furthermore, the potential synergistic effects of mixing these plant extracts with typical antibiotics like metronidazole have also just been briefly studied in only a few studies [16]. Because the bulk of research so far have focused on the effects of individual plant extracts, there is a big gap in our understanding of the potential benefits of a combined therapeutic strategy [17].

The goal of the current study was to determine if these extracts might effectively treat *P. gingivalis* whether used alone or in combination with Metronidazole. Additionally, the impact of these extracts on AHL synthesis and biofilm formation was explored. This approach has the potential to significantly contribute to the creation of more effective choices for treating periodontal disease, and this research may be the first step towards that direction.

2. Materials and Methods

2.1. Isolation and Culturing of Bacteria

The primary bacterium for this study, *P. gingivalis* (standard strain 33277), was procured from the American Type Culture Collection (ATCC). Additionally, clinical isolates of *P. gingivalis* were obtained from diagnosed patients at local dental clinics in collaboration with local health authorities. Further details of the clinical isolate collection procedure can be found in [21]. The bacteria were grown anaerobically at 37°C in in a brain-heart infusion agar medium (BHI-A) supplemented with hemin and vitamin k [22] as shown in Figure 1. The process involved in obtaining clinical isolate was conducted following the approval of the Local Ethical Committee (Ethical approval Reference number 382). All participants provided informed consent, and the study was performed in accordance with the Declaration of Helsinki.

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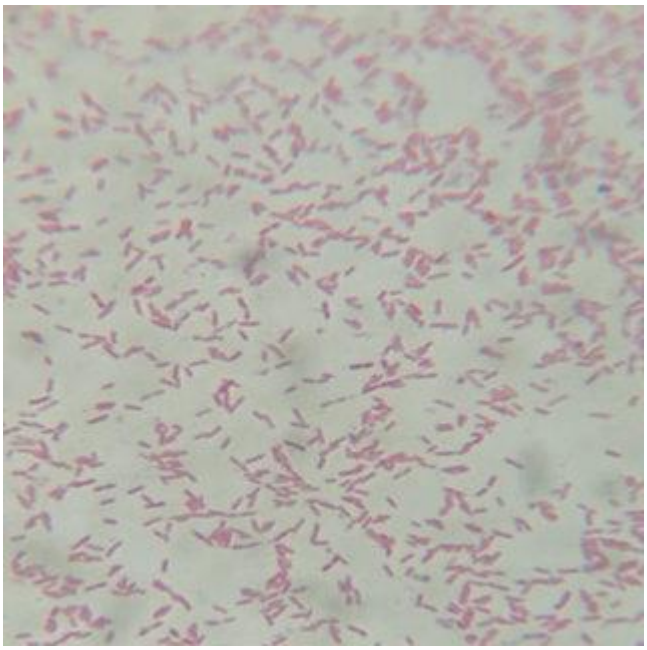
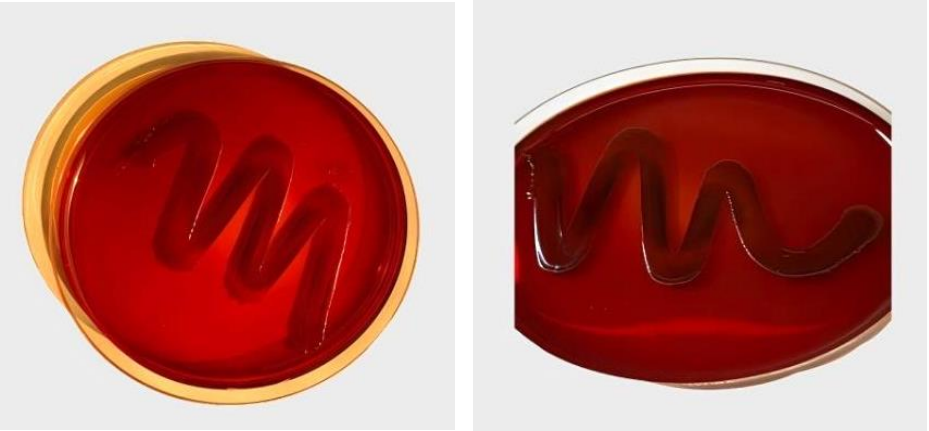


Figure 1. *P. gingivalis* appeared as Gram-negative coccobacilli under ×100 light microscope.

Detection of biofilm production by both the strains of *P. gingivalis* was assessed qualitatively by Congo Red Agar (CRA) media which composes of Brain heart infusion broth (37 gms/L), sucrose (50 gms/L), agar no.1 (10 gms/L) and congo red stain (0.8 gms/L, Himedia) [23]. The appearance of black stain indicated strong ability of quality biofilm formation (Figure 2). The clinical isolates were largely similar to those of the standard strain. Furthermore, 3 out 3 standard strains formed biofilm while 10 out of 11 clinical isolates form biofilm. This indicates that both the strains have good quality to form biofilm.



(**a**) (**b**)

Figure 2. Biofilm formation of *P. gingivalis* by Congo red agar (Qualitative method) (a) standard strain and (b) clinical isolate.

2.2. Supplement of Plant Extracts

Plant materials from Symphytum officinale (Comfrey) and Panax ginseng were sourced from certified vendors (Rejuvica Health, Gilbert, AZ, USA). The base concentrations for Comfrey, ginseng and Metronidazole were 330 mg/ml, 1000mg/ml and 500 mg/mL for without any alcohol or additive materials. Workable dilutions are prepared from the base concentrations by dissolving in Dimethyl sulfoxide (DMSO) and using systematic two-fold serial dilution technique. Eight dilutions were prepared for a G+S and G+S+F according to Table 1.

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Table 1. Total concentrations of plant extracts and Metronidazole.

Dilution	Total concentrations (mg/mL)	
	G+S	G+S+F
C_{D0}: Control	266	153.2
D1: first dilution	133	76.6
D2: second dilution	66.5	38.3
D3: third dilution	33.25	19.15
D4: fourth dilution	16.625	9.575
D5: fifth dilution	8.312	4.787
D6: sixth dilution	4.156	2.393
D7: seventh dilution	2.078	1.196
D8: eighth dilution	1.039	0.598

Total combination concentration of G+S (c3) was determined by using Equation 1.

$$v1 \times c1 + v2 \times c2 = v3 \times c3 \quad (1)$$

where v1/v2 is 1.0 mL as the volume of a single element (G or S), c1/c2 is the base concentration of a single element, and v3 is the combined volume of 5 mL. Volume of G or S is given as v1 = v2 = 1 mL, the baseline concentration of G, c1 = 1000 mg/mL, the baseline concentration of S, c2 = 330 mg/mL and the combined volume v3 = 5 ml, and the combined concentration of G+ S is calculated to be 266 mg/ml according to the above equation. Based on a two-fold serial dilution, half of this concentration (D1: first dilution for G+S) is 133 mg/mL.

Total combination concentration G+S+F (c3) was determined by using the same equation. Here v1/v2 is 1.0 mL as volume of a single element (G + S or F), c1/c2 is base concentration of a single element, and v3 is combination volume of 5 mL. Volume of G + S or F, v1 = v2 = 1 mL, base concentration of G+S, c1 = 266 mg/mL, base concentration of F, c2 = 500 mg/mL, and combination volume v3 = 5 ml were considered, and the combination concentration is calculated as 153.2 mg/mL. According to the two-fold serial dilution, half of this concentration (D1: first dilution for G+S+F) is 76.6 mg/mL.

2.3. Antibacterial Susceptibility Test

The antibacterial properties of the extracts from the plants and metronidazole, a common antibiotic used for treating infections by *P. gingivalis*, was assessed using the agar disk diffusion method. The test was performed by inoculating the bacteria on Brain Heart Infusion (BHI)-A plates supplemented with hemin and vitamin k to grow the bacteria. Subsequently, Mueller Hinton Agar (MHA) impregnated with different concentrations of plant extracts, metronidazole, and a combination of both extracts and metronidazole were placed onto the inoculated plates, which were then incubated anaerobically at 37°C for 48 hours. Zones of inhibition were measured to determine antibacterial activity (Figure 3).

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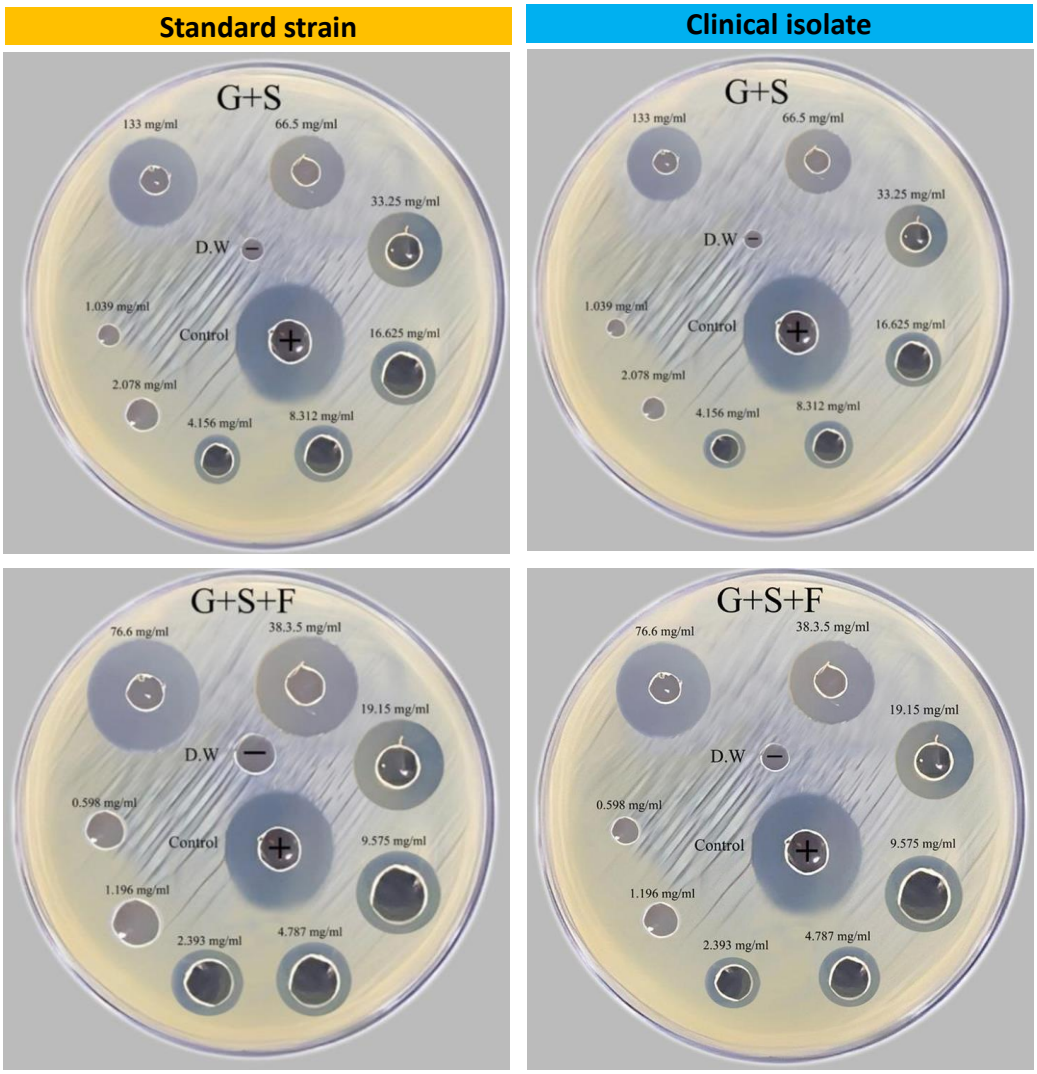

Figure 3. Inhibition zones created by (G+S) and (G+S+F) in competition with the standard strain and clinical isolate. of *P. gingivalis.*

2.4. Minimum Inhibitory Concentration (MIC) determination protocol

By making successive dilutions of the plant extracts and metronidazole, the MIC was determined. Individual wells or tubes containing the *P. gingivalis* inoculum received various dosages of the test chemicals. The samples were then placed in an incubator with the right conditions (5% H_2, 10% CO_2, and 85% N_2 at 37 °C) to promote bacterial growth. The lowest dose of each test chemical that prevented discernible bacterial growth was determined by visually inspecting the plates or tubes after incubation. The MIC value was regarded as being at this concentration.

2.5. Biofilm Inhibition and Disruption Assay

Using a microtiter plate biofilm test, the capacity for biofilm formation by *P. gingivalis* was evaluated in the presence and absence of plant extracts and metronidazole. Both the strains of *P. gingivalis* were suspended in BHI containing 1% glucose and adjusted to 0.5 McFarland (1.5×10^8 CFU/mL). Inoculate 20 µL of the bacterial suspension and 180 µL of BHI supplemented with 1% glucose into sterile flat-bottomed polystyrene microtiter plates [24]. The microplate was then incubated anaerobically at 37°C for 24 hours, and the degree to which the biofilm was inhibited was assessed using the crystal violet staining [25]. Then, the microplate was spectrophotometrically (UV-V, Germany) analyzed with a microplate reader to determine the optical density (OD). If the OD was

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greater than 0.240, the strain would be considered strong, moderate if the OD was between 0.120 and 0.240, and weak if the OD was less than 0.120 [26].

2.6. Quorum Sensing Inhibition Assay

The potential of the plant extracts to block the activity of quorum sensing was evaluated using a colorimetric method that produces orange coloration when combined with AHL, a molecule that is used as a quorum sensing tool. The reporter strain was cultivated alongside *P. gingivalis* in the presence and absence of the plant extracts. The degree of orange coloration was used as a means of measuring the activity of AHL, which was accomplished spectrophotometrically. The isolated organisms were considered to have a weak or negative association with AHLs if the OD was less than 0.98, and a strong or positive association with AHLs if the OD was greater than 0.98. [27].

2.7. Statistical Analysis

All experiments were performed in triplicate, and the data were expressed as mean ± standard deviation. Statistical analyses were performed using one-way ANOVA, followed by Tukey's multiple comparison tests. P values < 0.05 were considered statistically significant.

3. Results

3.1. Antibacterial Activity of Plant Extracts and Metronidazole

The antibacterial activities of both G+S and G+S+F against *P. gingivalis* in terms of inhibition zone diameters are presented in Table 2 and Figure 4. The mean inhibition-zone diameter increased with increasing concentrations of plant extracts. For (G+S), the zones of inhibition against strain ATCC (33277) ranged from 24.341±0.593 mm at 266 mg/mL to 13.022±0.013 at 4.156 mg/mL and against the clinical isolate, they ranged from 22.654±0.534mm at 266 mg/mL to 12.301±0.060 at 4.156 mg/mL. For (G+S+F), the zones of inhibition against the standard strain ranged from 25.000±0.001 mm at 153.2 mg/mL to 13.502±0.011 mm at 1.196 mg/mL and against the clinical isolate they ranged from 23.712±0.004 mm at 153.2 mg/mL to 12.533±0.033 mm at 1.196 mg/mL.

Table 2. Antibacterial activities of plant extracts and Metronidazole against *P. gingivalis*.

Microorganisms	Concentration	Mean inhibition zone diameter ± SD (mm)		T-test	P-value
		G+S	G+S+F		
Standard strain	C_{D0}	24.341±0.593	25.000±0.001	13.618	0.000
	D1	22.247±0.382	23.154±0.030		
	D2	19.503±0.205	20.221±0.012		
	D3	17.136±0.361	19.503±0.020		
	D4	15.381±0.146	17.970±0.645		
	D5	14.500±0.004	16.005±0.001		
	D6	13.022±0.013	14.302±0.620		
	D7	0.0	13.502±0.011		
	D8	0.0	0.0		
Clinical isolate	C_{D0}	22.654±0.534	23.712±0.004	12.796	0.000
	D1	21.254±0.125	22.0189±0.013		
	D2	19.105±0.010	19.5890±0.013		
	D3	17.071±0.004	18.3801±0.074		
	D4	14..791±0.015	16.155±0.033		
	D5	14.123±0.105	15.311±0.123		
	D6	12.301±0.060	14.002±0.001		
	D7	0.0	12.533±0.033		
	D8	0.0	0.0		

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Note: C_{D0} is control group concentration; D1 to D8 is 2-fold serial dilution (Table 1); 0.0 means no inhibition zone; Panax Ginseng and Symphytum Officinale (G+S), Panax Ginseng. Symphytum Officinale and metronidazole (G+S+F).

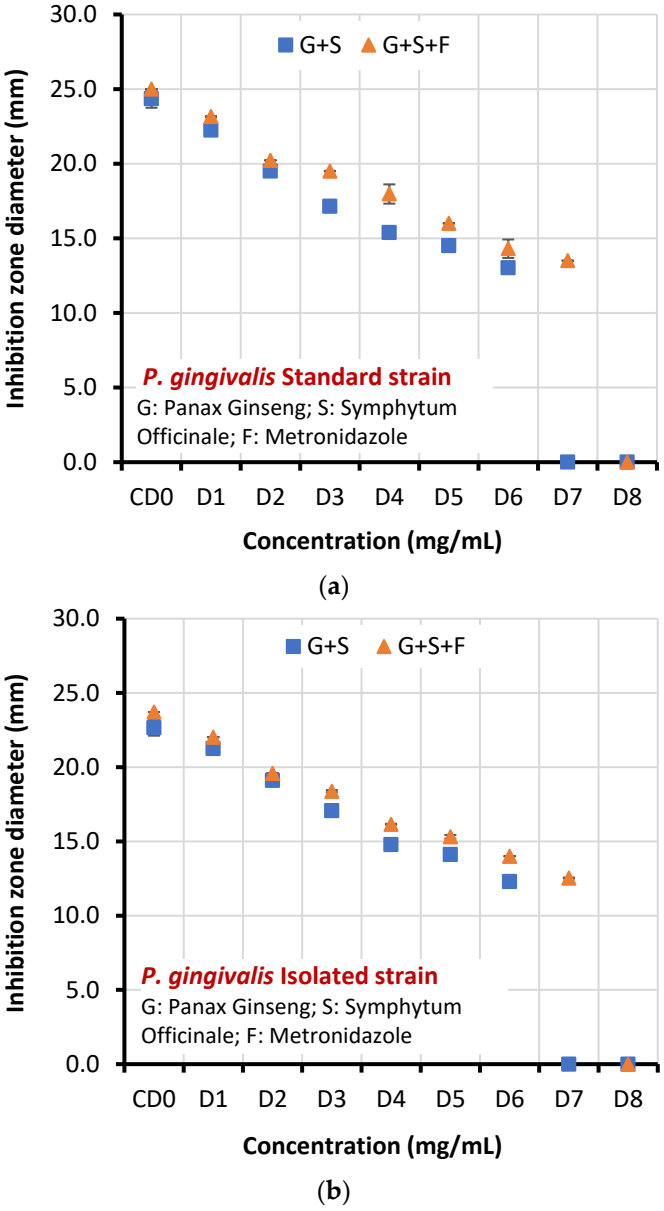

Figure 4. The inhibitory zones for bacteria, which are created by plant extracts and metronidazole at different concentrations against the two types of *P. gingivalis* (**a**) the standard strain and (**b**) the clinical isolate.

When the plant extracts were used in combination with Metronidazole, a noticeable enhancement of antibacterial activity was observed. The zones of inhibition increased significantly, suggesting a synergistic effect. For example, when Panax ginseng (1000 mg/ml) Symphytum officinale (330 mg/ml) and Metronidazole (500 mg/ml) were combined (G+S+F), the zones of inhibition were 25.000±0.001 mm and 23.712±0.004 mm against the standard strain and clinical isolate, which were significantly greater ($P<0.05$) than the zones produced by extracts alone (G+S).

3.2. MIC determination

The mean MICs of both G+S and G+S+F against *P. gingivalis* of the standard strain and the clinical isolate are listed in Table 3.

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Table 3. MIC against *P. gingivalis* (standard strain and clinical isolate).

Microorganisms	Minimum Inhibitory Concentration (MIC) mg/mL (Doses)	
	G+S	G+S+F
Standard strain	8.312 (D5)	2.393 (D6)
Clinical Isolate	8.312 (D5)	2.393 (D6)

3.3. Biofilm disruption of P. gingivalis

After being impacted by several experimental extracts, the effectiveness of these extracts in inhibiting biofilms formed by *P. gingivalis* was assessed. The antibacterial activity of the extracts tested was demonstrated by a significant difference in the optical density of the biofilms in the various treatments ($p < 0.05$) (Table 4 and Figure 5). It is important to recognize that even the D8 (lowest concentration) had some antibacterial properties. For both the strains, the OD values without any extracts and Metronidazole were higher than 0.240 (CB: 3.315 and 3.722), hence the strains are considered strong.

Biofilm disruption results showed that established biofilms were also sensitive to the extracts. G+S at a concentration of D5: 8.312 mg/mL disrupted about 59.36% and 52.55% of the established biofilm for the standard strain and clinical isolate respectively (Table 5). On the other hand, G+S+F at a concentration of D6: 2.393 mg/mL disrupted about 58.64% and 52.12% of the established biofilm for the standard strain and clinical isolate respectively**.** These concentrations were considered as the minimum dose that inhibits the bacteria.

Table 4. Mean optical density (OD) of the biofilm formed by *P. gingivalis*.

Microorganism	Concentration	Mean optical density (OD) of biofilm ±SD		T-test	P-value
		G+S	G+S+F		
Standard strain	C_B	3.315±0.098	3.315±0.098	3.859	0.005
	D1	0.074±0.004	0.051±0.022		
	D2	0.157±0.070	0.094±0.002		
	D3	0.579±0.069	0.211±0.021		
	D4	1.109±0.037	0.466±0.015		
	D5	1.347±0.081	1.144±0.010		
	D6	1.727±0.047	1.371±0.133		
	D7	2.043±0.092	1.980±0.112		
	D8	2.921±0.14	2.513±0.187		
Clinical isolate	C_B	3.722±0.069	3.722±0.069	4.402	0.002
	D1	0.090±0.145	0.080±0.001		
	D2	0.411±0.001	0.155±0.081		
	D3	0.820±0.040	0.334±0.113		
	D4	1.591±0.005	0.571±0.008		
	D5	1.766±0.208	1.530±0.041		
	D6	2.189±0.050	1.782±0.034		
	D7	2.900±0.103	2.085±0.068		
	D8	3.135±0.022	2.870±0.011		

Note: C_B is control group with biofilm without any extract; D1 to D8 is 2-fold serial dilution (Table 1); Panax Ginseng and Symphytum Officinale (G+S), Panax Ginseng + Symphytum Officinale+ Metronidazole (G+S+F).

Table 5. Percentage inhibition of biofilm formation by *P. gingivalis* (strain ATCC 33277) and isolate.

Microorganism	Concentration	Percentage inhibition of biofilm formation [(1-(OD with dilution/OD with control))×100]

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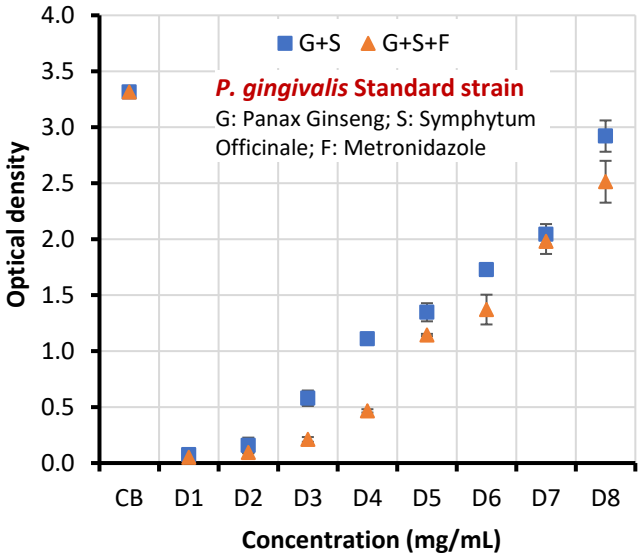
		G+S	G+S+F
Standard strain	D1	97.76	98.46
	D2	95.26	97.16
	D3	82.53	93.63
	D4	66.54	85.94
	D5	59.36	65.49
	D6	47.90	58.64
	D7	38.37	40.27
	D8	11.88	24.19
Clinical isolate	D1	97.58	97.85
	D2	88.95	95.83
	D3	77.96	91.02
	D4	57.25	84.65
	D5	52.55	58.89
	D6	41.18	52.12
	D7	22.08	43.98
	D8	15.77	22.89
X^2		3.814	
p value		0.00021	



(**a**)

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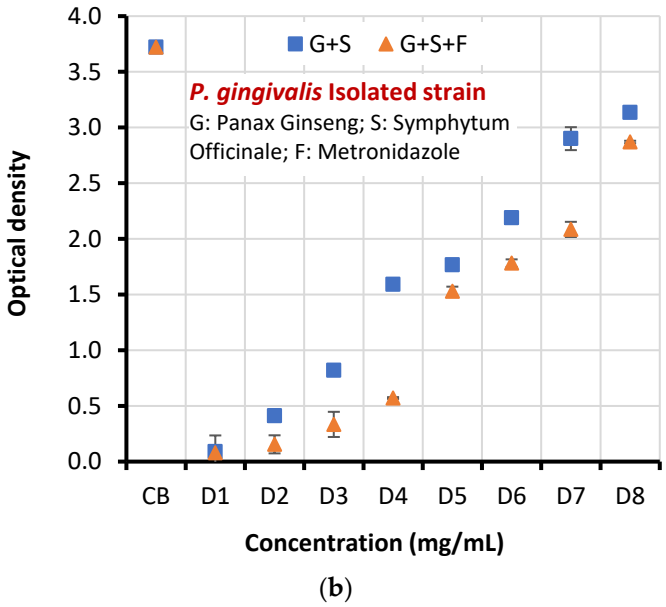

(**b**)

Figure 5. Biofilm inhibition activity (optical density) at different does against *P. gingivalis* (**a**) standard strain and (**b**) clinical isolate.

3.4. Quorum Sensing Inhibition

Combination of Symphytum officinale and Panax ginseng (G+S) significantly reduced the production of AHLs, as indicated by a decrease in orange pigmentation in the reporter strain (Table 6 and Figure 6). A dose-dependent decrease in AHL production was observed with increasing concentrations of both extracts (Table 7). With S+G at the concentration D5: 8.312 mg/mL reduced AHL production by approximately 67.55% and 55.76 mg/mL for the standard strain and clinical isolate respectively. While with G+S+F at a concentration of D6: 2.393 mg/ml disrupted about 55.62% and 51.53% of the established biofilm for the standard strain and clinical isolate respectively**.** These concentrations were considered as the minimum amount of dose that inhibit the bacteria.

The data strongly suggest that both plant extracts, especially when used in combination with Metronidazole, not only show antibacterial properties but also inhibit biofilm formation and quorum sensing against *P. gingivalis*. The ability of these extracts to disrupt established biofilms further augments their potential as promising therapeutic agents for the control of periodontal infections caused by *P. gingivalis*.

Table 6. Influence of plant extracts and Metronidazole on AHLs production.

Microorganism	Concentration	AHL activity ± SD		T-test	P-value
		G+S	G+S+F		
Standard strain	C_B	2.826±0.037		3.264	0.011
	D1	0.006±0.110	0.003±0.001		
	D2	0.130±0.033	0.109±0.110		
	D3	0.290±0.031	0.201±0.122		
	D4	0.709±0.001	0.506±0.004		
	D5	0.917±0.025	0.898±0.100		
	D6	1.327±0.039	1.254±0.062		
	D7	1.524±0.135	1.332±0.004		
	D8	2.310±0.078	2.187±0.122		
Clinical isolate	C_B	3.151±0.098		3.204	0.013
	D1	0.017±0.002	0.000		
	D2	0.152±0.011	0.111±0.120		
	D3	0.331±0.003	0.201±0.098		

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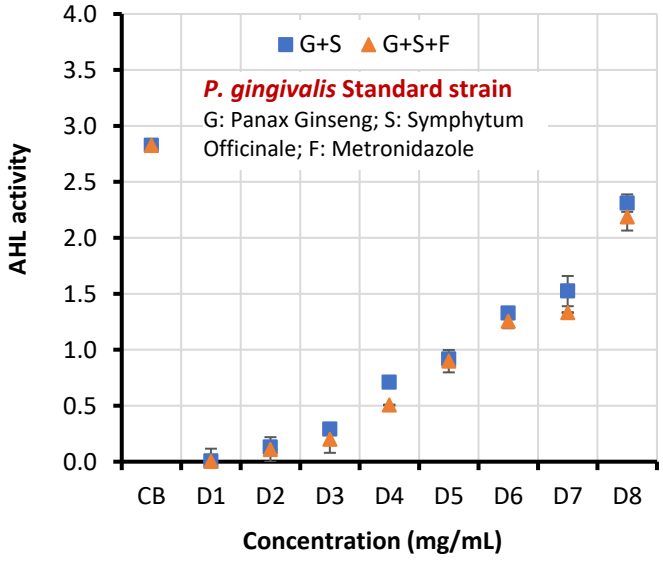
	D4	0.901±0.014	0.709±0.013
	D5	1.394±0.010	1.229±0.020
	D6	1.787±0.009	1.527±0.058
	D7	1.915±0.143	1.847±0.101
	D8	2.397±0.060	2.215±0.029

Note: C_B is control group with biofilm without any extract; D1 to D8 is 2-fold serial dilution (Table 1); Panax Ginseng and Symphytum Officinale (G+S), Panax Ginseng + Symphytum Officinale+ Metronidazole (G+S+F).

Table 7. Percentage inhibition of Acylated Homoserine Lactones (AHLS) activity by *P. gingivalis* (strain ATCC 33277) and isolate.

Microorganism	**Concentration**	**Percentage inhibition of AHL activity [(1-(AHL with dilution/AHL with control))×100]**	
		G+S	G+S+F
Standard strain (ATCC 33277)	D1	99.78	99.89
	D2	95.39	96.14
	D3	89.73	92.88
	D4	74.91	82.09
	D5	67.55	68.22
	D6	53.04	55.62
	D7	46.07	52.86
	D8	18.25	22.61
Clinical isolate	D1	99.46	100
	D2	95.17	96.47
	D3	89.49	93.62
	D4	71.40	77.49
	D5	55.76	60.99
	D6	43.28	51.53
	D7	39.22	41.38
	D8	23.92	29.70
X^2		3.291	
p value		0.00013	



(**a**)

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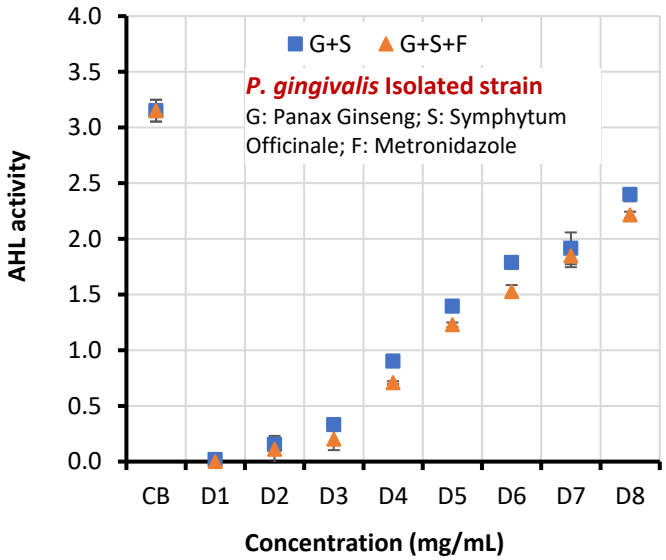

(**b**)

Figure 6. Acylated Homoserine Lacticones (AHLs) activity to inhibit the growth of *P. gingivalis* in a dose-dependent manner (**a**) standard strain and (**b**) clinical isolate.

4. Discussion

The results of the current investigation showed that the extracts of Panax ginseng and Symphytum officinale had significant antibacterial actions against *P. gingivalis*, limiting the generation of quorum-sensing molecules and preventing the creation of new biofilms [1]. They demonstrated a synergistic interaction with the antibiotic Metronidazole, greatly boosting its antibacterial effectiveness [2]. This information may be significant because using plant extracts [28] in conjunction with antibiotics may help to combat the rising issue of antibiotic resistance [3].

The effectiveness of the plant extracts against both the standard and clinical isolates of *P. gingivalis* points to the possibility of using them as therapeutic agents to treat periodontal disorders [29], [30]. It is crucial to keep in mind that these results are preliminary and more research is required to completely comprehend the mechanisms of action of these plant extracts and to confirm their efficiency in vivo [4].

In particular, those connected to biofilm formation and quorum sensing, the study offers encouraging insights into the potential of plant-derived chemicals in creating more efficient methods for managing *P. gingivalis* infections. Future research should [31], [32], however, focus on identifying the precise bioactive substances that are in charge of these antibacterial activities as well as assessing the effectiveness and safety of these extracts in human clinical trials [5]. Additionally, research into additional plant-derived substances with comparable qualities may result in the identification of brand-new antibacterial substances that might significantly improve the capacity to manage periodontal disorders [6].

According to the current study's findings, Panax ginseng and Symphytum officinale both have strong antibacterial activity against *P. gingivalis*, both in terms of preventing growth and dissolving existing biofilms [7]. The bacteria that were isolated from patients were more active and energetic than the conventional strain, as evidenced by the smaller size of the inhibition zone (Table 2), increased optical density (Table 4), and increased activity of AHL (Table 6).This also corroborates with other studies that claim clinical isolates may display unique characteristics [11]–[13]. Additionally, there was increasing in inhibition zone diameter by increasing extracts concentrations and synergy when Metronidazole was combined with plant extracts (Table 2), suggesting a possible method for boosting the effectiveness of already prescribed antibiotics [8].

This work provides new insights into a growing body of research supporting the use of natural extracts for enhanced antimicrobial effectiveness when compared to our previous study (21). Where inhibition zone diameter of G+S (24.341±0.593) was greater than G, S, or F alone (G= 20.5120 ± 0.014,

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S=18.6111 ± 0.147, F= 20.4119 ± 0.114) and more synergistic effects were observed when combining the extracts with metronidazole, the inhibition zone of G+S+F (25.000±0.001) was greater than that of G+F (G+F=24.0125 ± 0.321) or S+F (22.0367 ± 0.014).

Additionally, both plant extracts shown a notable capacity to suppress the synthesis of AHLs, a crucial quorum sensing molecule involved in *P. gingivalis* biofilm development [9]. This is particularly intriguing since it shows that these plant extracts may not only directly kill bacteria but also interfere with the intricate networks of communication that *P. gingivalis* use to coordinate the creation of biofilms and other virulence traits [10].

The bioactive substances responsible for the reported antibacterial activity have not yet been identified, despite these encouraging results. Because Symphytum officinale and Panax ginseng are both known to contain a range of bioactive substances [14], [15], the antibacterial effects that have been reported may be the result of one or more of these substances working alone or in combination [11].

The safety and effectiveness of these plant extracts must also be examined in clinical studies [33], [34] before they can be taken into consideration for therapeutic application, despite the fact that the in vitro results are encouraging [13]. Additionally, since the current study only looked at two plant species, investigating additional plant-derived substances with comparable qualities may result in the identification of brand-new antibacterial chemicals, considerably boosting the ability to treat periodontal diseases [14], [19], [21], [35].

In particular, those connected to biofilm formation and quorum sensing, the study offers encouraging insights into the potential of plant-derived chemicals [16]–[18] in creating more efficient methods for managing *P. gingivalis* infections. Future research should, however, focus on identifying the precise bioactive substances that are in charge of these antibacterial activities as well as assessing the effectiveness and safety of these extracts in human clinical trials [14].

Future study should concentrate on in vivo confirmation of the efficacy of Panax ginseng and Symphytum officinale in relation to *P. gingivalis*, building on the current findings. It would also be helpful to investigate any potential negative effects or toxicities connected with these extracts, particularly when used over an extended period of time. In order to create new, more potent medications to treat *P. gingivalis*, it is crucial to pinpoint the precise chemical components in these extracts that are responsible for the antibacterial action.

Understanding the methods by which these plant extracts prevent *P. gingivalis* from forming biofilms and using quorum sensing might also provide light on the pathogenicity of this bacterium and the larger significance of these processes in periodontal disease. It could also result in the creation of more specialised and successful treatment approaches.

5. Conclusions

This study assessed the effectiveness of Panax ginseng and Symphytum officinale, two plant-derived substances, in reducing periodontal infections caused on by *P. gingivalis* bacteria. The production of AHLs, a key quorum sensing molecule in *P. gingivalis*, decreased in the presence of the extracts and they showed inhibitory effects on the development of biofilms as well as destruction of already-formed biofilms. Curiously, a synergistic effect was seen when Metronidazole was coupled with these plant extracts, further increasing their antibacterial activity against *P. gingivalis*. Plant Extracts and Metronidazole showed a higher zone of inhibition, lower optical density and lower AHL production compared to the plant extracts alone at all tested concentrations affirming their synergistic effect**.** Given the problems with antibiotic resistance, plant extracts or their combination with Metronidazole will open a new avenue for treating periodontal disease and improve the quality of life.

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