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RESEARCH ARTICLE

Antibacterial Activity of the Ethanol Extract of *Paeonia Lactiflora* on Growth of Oral Bacteria

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Abstract

The roots of *Paeonia lactiflora* Pallas are important crude drugs in Korean traditional medicine. A decoction of the dried root has been used in the treatment of rheumatoid arthritis, systemic lupus erythematosus, dysmenorrhea, hepatitis, muscle cramping, inflammation, spasms, and fever and stimulate blood circulation. In this study, the combination effect of the ethanol extract of *Paeonia lactiflora* (PLE) was evaluated against oral bacteria, either alone or with antibiotics, via broth dilution method and checkerboard and time kill assay. In these results, MIC/MBC values for PLE against all the tested bacteria ranged between 250-2000/250-2000 microg/mL, for ampicillin 0.031-16/0.125-32 microg/mL, gentamicin 2-256/4-512 microg/mL, erythromycin 0.008-32/0.016-64 microg/mL, and vancomycin 0.5-64/1-128 microg/mL, respectively. Furthermore, the MIC and MBC were reduced to one half-four as a result of the combination of PLE with antibiotics. Six hours of treatment with 1/2 MIC of PLE with 1/2 MIC of antibiotics resulted from an increase of the rate of killing in units of CFU/mL to a greater degree than was observed with alone. These results suggest that the ethanol extract of *Paeonia lactiflora* (PLE) is important in the antibacterial actions of oral pathogen agents.

Keywords: *Paeonia lactiflora* Pallas; Antibacterial activity; Oral pathogen bacteria; Synergistic effect; Minimum inhibitory concentrations (MICs); Minimum bactericidal concentrations (MBCs)

List of abbreviations: PLE: the ethanol extract of *Paeonia lactiflora* Pallas; MICs: Minimum inhibitory concentrations; MBCs: Minimum bactericidal concentrations; CFU: Colony Forming Unit; FIC index: Fractional Inhibitory Concentration; FBC index: Fractional Bactericidal Concentration index

Introduction

More than 700 different bacterial species have been detected in the oral cavity of humans [1]. Saliva contains 10^8 to 10^9 bacteria per milliliter, and some of these adhere to the teeth and initiate formation of a dental biofilm, previously called dental plaque [1]. Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable global infectious diseases [2]. Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases [3]. The development of dental caries involves acidogenic and aciduric gram-positive bacteria, primarily the mutans streptococci (*Streptococcus mutans* and *S. sobrinus*), lactobacilli, and actinomycetes, which metabolize sucrose to organic acids (mainly lactic acid) that dissolve the calcium phosphate in teeth, causing decalcification and eventual decay [2,4].

Periodontal disease results from chronic infection and inflammation of the tissues that support the teeth [2,5]. This oral inflammatory disease is driven by pathogenic succession characterized by a shift in microbial species in the gingival sulcus from gram-positive, facultative and fermentative microorganisms to predominantly gram-negative, anaerobic, chemo-organotrophic, and proteolytic microorganisms [5,6]. Periodontal disease also is implicated in several systemic diseases, including preterm/low birth weight deliveries, cardiovascular events, diabetes and other systemic conditions [5-7].

Several antibacterial agents including, fluorides, phenol derivatives, ampicillin, erythromycin, penicillin, tetracycline, and vancomycin have been used widely in dentistry to inhibit bacterial growth [8,9]. However, excessive use of these chemicals can result in derangements of the oral and intestinal flora and cause side effects such as microorganism susceptibility, vomiting, diarrhea and tooth staining [10]. These problems necessitate further search for natural antibacterial agents that are safe for humans and specific for oral pathogens. Natural products have recently been investigated more thoroughly as promising agents to prevent oral diseases, especially plaque-related diseases such as dental caries [11,12].

Paeonia lactiflora Pallas belongs to the genus Paeonia in the family Paeoniaceae, which is an important crude drug in Korean traditional medicine [13]. A decoction of the dried root without bark has been used in the treatment of rheumatoid arthritis, systemic lupus erythematosus, hepatitis, dysmenorrheal, muscle cramping, inflammation, and spasms, and fever for centuries [13-15]. Monoterpene glycosides, such as albiflorin, oxypaeoniflorin, and paeoniflorin, have been isolated from *P. lactiflora* roots with galloyl and phenolic compounds, including benzoic acid, catechin, gallic acid, methyl gallate, paeonol, and 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucose (PGG) [16]. These compounds have been revealed to have anti-inflammatory, anti-diabetic, anti-oxidant, anti-allergic, anti-coagulative, sedative, analgesic acitivities, and etc. Paeoniflorin, a major constituent of *Paeoniae Radix*, has been reported to exhibit diverse biological activities, including anti-inflammatory, spasmolytic, immune-regulating, and gastroprotective activities [17,18].

Phytotherapy has many potentially significant advantages associated with the synergistic interactions like, increased efficiency, reduction of undesirable effects, increase in the stability or bioavailability of the free agents and obtaining an adequate therapeutic effect with relatively small doses, when compared with a synthetic medication [19]. Recently, plant antimicrobials have been found to be synergistic enhancers in that though they may not have any antimicrobial properties alone, but when they are taken concurrently with standard drugs they enhance the effect of that drug [20]. Although, plant derived antimicrobial are less potent, this enhances the need to research a synergism interaction between plant bioactive products and antimicrobial agent. Therefore, the association between plant extracts and synthetic drugs has shed light to a novel approach in controlling multidrug resistant strains and in modulating the action of antibiotics [19,20].

The aim of the present study was to examine antibacterial effect of *Paeonia lactiflora* extract (PLE) against oral pathogens and to investigate the synergy between PLE and commonly used antibiotics.

Material and Methods

Plant material and preparation of 50% ethanol extract of *Paeonia lactiflora* extract (PLE)

Dried flowers from *P. lactiflora* (PLE, 2 kg) were macerated and extracted three times with 50% EtOH (10 L) for 4 h at 80 °C. The combined 50% EtOH extract (30 L) was clarified by filtration and evaporated to obtain brown syrup (300 g). One hundred mg/mL of extract was dissolved in 10% dimethyl sulfoxide (DMSO, Sigma Chemical Co., St. Louis, MO, USA) and then, diluted with bacteria culture medium for testing. All of the extract was kept at 4 °C in the dark until further use.

Bacterial strains

The oral bacterial strains used in this study were: cariogenic bacterial strains, *Streptococcus mutans* ATCC 25175 (American Type Culture Collection), *Streptococcus sanguinis* ATCC 10556, *Streptococcus sobrinus* ATCC 27607, *Streptococcus ratti* KCTC (Korean Collection for Type Cultures) 3294, *Streptococcus criceti* KCTC 3292, *Streptococcus parasanguinis* KCOM 1497 (Korean Collection for Oral Microbiology), *Streptococcus downei* KCOM 1165, *Streptococcus anginosus* ATCC 31412, and *Streptococcus gordonii* ATCC 10558 and periodontopathogenic bacterial strains, *Actinobacillus actinomycetemcomitans* ATCC 43717, *Fusobacterium nucleatum* ATCC 10953, and *Porphylomonas gingivalis* ATCC 33277. Brain-Heart Infusion (BHI) broth supplemented with 1% yeast extract (Difco Laboratories, Detroit, MI) was used for cariogenic bacterial strains (facultative anaerobic bacteria). For periodontopathogenic bacterial strains (microaerophilic and obligate anaerobic bacteria), BHI broth containing hemin 1 μg/mL (Sigma, St. Louis, MO, USA) and menadione 1 μg/mL (Sigma) was used.

Minimum inhibitory concentrations/minimum bactericidal concentrations assay

The minimum inhibitory concentrations (MICs) were determined for PLE by the broth dilution method, and were carried out in triplicate. The antibacterial activities were examined after incubation at 37 °C for 18 h (facultative anaerobic bacteria), for 24 h (microaerophilic bacteria), and for 1-2 days (obligate anaerobic bacteria) under anaerobic conditions. The inoculums suspension containing 0.125-4 mg/mL PLE was added to give a final concentration of between 5×10^6 and 8×10^6 CFU/mL for the assays. MICs were determined as the lowest concentration of test samples that resulted in a complete inhibition of visible growth in the broth. MIC_{50}s and MIC_{90}s, defined as MICs at which, 50 and 90%, respectively of oral bacteria were inhibited, were determined. Following anaerobic incubation of MICs plates, the minimum bactericidal concentrations (MBCs) were determined on the basis of the lowest concentration of PLE that kills 99.9% of the test bacteria by plating out onto each appropriate agar plate. Ampicillin, gentamicin, erythromycin, and vancomycin (Sigma) were used as standard antibiotics in order to compare the sensitivity of PLE against oral bacteria.

Checker-board dilution test

The antibacterial effects of a combination of PLE, which exhibited the highest antimicrobial activity and antibiotics, were assessed by the checkerboard test as previously described [21]. The antimicrobial combinations assayed included PLE with ampicillin, gentamicin, erythromycin, and/or vancomycin. Serial dilutions of two different antimicrobial agents were mixed in cation-supplemented Mueller-Hinton broth. After 24-48 h of incubation at 37 °C, the MICs were determined to be the minimal concentration at which there was no visible growth and MBCs were determined on the basis of the lowest concentration of PLE that kills 99.9% of the test bacteria by plating out onto each appropriate agar plate. The fractional inhibitory concentration (FIC)/fractional bactericidal concentration (FBC) index was calculated according to the equation: FIC/FBC index=FIC/FBC_A+FIC/FBC_B= (MIC/MBC of drug A in combination/MIC/MBC of drug A alone)+(MIC/MBC of drug B in combination/MIC/MBC of drug B alone). The FIC and FBC index are the sum of the FICs and FBCs of each of the drugs, which in turn is defined as the MIC and MBC of each drug when it is used in combination divided by the MIC and MBC of the drug when it is used alone. The interaction was defined as synergistic if the FIC and FBC index was less than or equal to 0.5, additive if the FIC and FBC index was greater than 0.5 and less than or equal 1.0, indifferent if the FIC and FBC index was greater than 1.0 and less than or equal to 2.0, and antagonistic if the FIC and FBC index was greater than 2.0 [21].

Time-kill curves

Bactericidal activities of the drugs under study were also evaluated using time-kill curves on oral bacteria. Tubes containing Mueller-Hinton supplemented to which antibiotics had been added at concentrations of the 1/2 MIC were inoculated with a suspension of the test strain, giving a final bacterial count between 5~8×10^6 CFU/mL. The tubes were thereafter incubated at 37 °C in an anaerobic chamber and viable counts were performed at 0, 0.5, 1, 2, 3, 6, 9, 12, 18 and 24 h after addition of antimicrobial agents, on agar plates incubated for up to 48 h in anaerobic chamber at 37 °C. Antibiotic carryover was minimized by washings by centrifugation and serial 10-fold dilution in sterile phosphate-buffered saline, pH 7.3. Colony counts were performed in duplicate, and means were taken. The solid media used for colony counts were BHI agar for streptococci and BHI agar containing hemin and menadione for *P. intermedia* and *P. gingivalis*.

Results and Discussion

The PLE was evaluated for their antimicrobial activities against twelve common bacterial species present in the oral cavity. The results of the antimicrobial activity showed that PLE exhibited antimicrobial activities against cariogenic bacteria (MICs, 250 to 1000 μg/mL; MBCs, 250 to 2000 μg/mL), against periodontopathogenic bacteria (MICs, 250 to 2000 μg/mL; MBCs, 500 to 2000 μg/mL) and for ampicillin, either 0.31/0.125 or 16/32 μg/mL; for gentamicin, either 2/4 or 256/512 μg/mL; for erythromycin, either 0.008/0.016 or 32/64 μg/mL; for vancomycin, either 0.5/1 or 64/128 μg/mL on tested all bacteria (Table 1). The range of MIC_{50} PLE was from 11 to 953 μg/mL. The PLE showed stronger antimicrobial activity against *S. gordonii* than other bacteria.

Samples	PLE			Ampicillin	Gentamicin	Erythromycin	Vancomycin
	$MIC_{50<}$	$MIC_{90<}$	MIC/MBC (μg/mL)	MIC/MBC (μg/mL)			
S. mutans ATCC 25175[1]	63	250	250/250	0.25/0.25	8/16	0.063/0.125	1/2
S. sanguinis ATCC 10556	76	500	500/1000	0.125/0.5	8/32	0.016/0.031	0.5/1
S. parasanguinis KCOM 1497[2]	46	1000	1000/1000	0.5/1	16/32	0.25/0.5	2/4
S. sobrinus ATCC 27607	132	500	500/1000	0.063/0.125	8/32	0.031/0.063	1/2
S. ratti KCTC 3294[3]	187	500	500/1000	0.25/0.5	8/16	0.008/0.016	1/1
S. criceti KCTC 3292	184	500	500/2000	0.031/0.125	16/32	0.125/0.25	2/4
S. downei KCOM 1165	29	1000	1000/1000	2/4	32/64	0.25/0.5	8/16
S. anginosus ATCC 31412	32	1000	1000/2000	0.063/0.25	8/16	0.25/0.5	1/4
S. gordonii ATCC 10558	11	250	250/250	0.125/0.25	16/32	0.031/0.063	0.5/1
A. actinomycetemcomitans ATCC 43717	95	2000	2000/2000	16/32	16/16	0.125/0.25	2/4
F. nucleatum ATCC 51190	82	500	500/1000	4/16	2/4	32/64	64/128

Samples	PLE			Ampicillin	Gentamicin	Erythromycin	Vancomycin
	$MIC_{50<}$	$MIC_{90<}$	MIC/MBC (μg/mL)	MIC/MBC (μg/mL)			
P. intermedia ATCC 49049	953	1000	1000/2000	1/2	32/64	16/32	16/36
P. gingivalis ATCC 33277	124	250	250/500	0.25/0.5	256/512	2/8	16/16

[1]American Type Culture Collection (ATCC)
[2]Korean collection for Oral Microbiology (KCOM)
[3]Korean collection for Type Cultures (KCTC)
Table 1: Antibacterial activity of 50% ethanol extract of Paeonia lactiflora pallas (PLE) and antibiotics in oral bacteria

Natural products are a major source of chemical diversity and have provided important therapeutic agents for many bacterial diseases [12,13,19,20]. Combinations of some herbal materials and different antibiotics might affect the inhibitory effect of these antibiotics [20,21]. It is always suggested to treat bacterial infections with a combination of antimicrobial agents for the prevention of drug resistance development and to improve efficacy. Drug combinations having synergistic interactions are generally considered as more effective and, therefore, preferable [19,20].

The synergistic effects of the PLE alone or with antibiotics were evaluated in oral bacteria (Table 2,3,4 and 5). In combination with MICs for ampicillin, PLE was reduced ≥4-fold in cariogenic bacteria, producing a synergistic effect as defined by FICI ≤ 0.5 except *S. sobrinus* and *S. anginosus*, and in all tested periodontopathogenic bacteria by FICI ≤ 0.5. The MBC for ampicillin was shown synergistic effects in *S. sanguinis*, *S. ratti*, and *S. criceti* by FBCI ≤ 0.5, and in the periodontopathogenic bacteria, *P. intermedia* by FBCI ≤ 0.5 (Table 2). In combination with MIC of gentamicin, the PLE was reduced ≥4-8-fold in all tested bacteria expect *S. mutans* and *S. downei* by FICI ≥ 0.75 and MBC in *S. mutans*, *S. sanguinis*, *S. ratti*, *S.downei*, *S. gordonii*, and *A. actinomycetemcomitans* by FBCI ≥ 0.75 (Table 3). In combination with MIC of erythromycin, the PLE was reduced ≥4-8-fold in all tested bacteria expect *S. sanguinis*, *S. sobrinus*, and *S. gingivalis* by FICI ≥ 0.75 and MBC in *S. sanguinis*, *S. sobrinus*, and *S. gordonii* by FBCI ≥ 0.75 (Table 4). In combination with MICs for vancomycin, PLE was reduced ≥4-fold in all tested bacteria, producing a synergistic effect as defined by FICI ≤ 0.5 except *S. parasanguinis*, *S. sobrinus*, and *S. criceti* by FICI ≥ 0.75, and MBC for vancomycin was shown synergistic effects in all tested bacteria except *S. sanguinis*, *S. parasanguinis*, *S. gordonii*, and *A. actinomycetemcomitans* by FICI ≥ 0.75 (Table 5).

Strains	Agent	MIC/MBC (μg/mL)		FIC/FBC	FICI/ FBCI[2]	Outcome
		Alone	Combination[1]			
S. mutans ATCC 25175[3]	PLE	250/250	63/125	0.25/0.5	0.5/1.0	Synergistic/ Additive
	Ampicillin	0.25/0.25	0.063/0.125	0.25/0.5		
S. sanguinis ATCC 10556	PLE	500/1000	125/250	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Ampicillin	0.125/0.5	0.031/0.125	0.25/0.25		
S. parasanguinis KCOM 1497[4]	PLE	1000/1000	250/500	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Ampicillin	0.5/1	0.125/0.25	0.25/0.25		
S. sobrinus ATCC 27607	PLE	500/1000	250/500	0.5/0.5	0.75/0.75	Synergistic/ Additive
	Ampicillin	0.063/0.125	0.016/0.031	0.25/0.25		
S. ratti KCTC 3294[5]	PLE	500/1000	125/250	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Ampicillin	0.25/0.5	0.063/0.125	0.25/0.25		
S. criceti KCTC 3292	PLE	500/2000	125/500	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Ampicillin	0.031/0.125	0.008/0.031	0.25/0.25		
S. downei KCOM 1165	PLE	1000/1000	250/500	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Ampicillin	2/4	0.5/1	0.25/0.25		
S. anginosus ATCC 31412	PLE	1000/2000	250/500	0.25/0.25	0.75/0.75	Additive/ Additive
	Ampicillin	0.063/0.25	0.031/0.125	0.5/0.5		
S. gordonii ATCC 10558	PLE	250/250	63/125	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Ampicillin	0.125/0.25	0.031/0.063	0.25/0.25		
A. actinomycetemcomitans ATCC 43717	PLE	2000/2000	500/1000	0.25/0.5	0.5/1.0	Synergistic/ Additive
	Ampicillin	16/32	4/16	0.25/0.5		
F. nucleatum ATCC 51190	PLE	500/1000	125/500	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Ampicillin	4/16	1/4	0.25/0.25		

Strains	Agent	MIC/MBC (μg/mL)		FIC/FBC	FICI/ FBCI[2]	Outcome
		Alone	Combination[1]			
P. intermedia ATCC 49049	PLE	1000/2000	250/250	0.25/0.125	0.5/0.375	Synergistic/ Synergistic
	Ampicillin	1/2	0.25/0.5	0.25/0.25		
P. gingivalis ATCC 33277	PLE	250/500	63/125	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Ampicillin	0.25/0.5	0.063/0.125	0.25/0.25		

[1]The MIC and MBC of 50% ethanol extract of *Paeonia lactiflora* pallas (PLE) with ampicillin
[2] The fractional inhibitory concentration index (FIC index)
[3]American Type Culture Collection (ATCC)
[4]Korean collection for type cultures (KCTC)
Table 2: Synergistic effects of 50% ethanol extract of *Paeonia lactiflora* pallas (PLE) with ampicillin against oral bacteria

Strains	Agent	MIC/MBC (μg/mL)		FIC	FICI[2]	Outcome
		Alone	Combination[1]			
S. mutans ATCC 25175[3]	PLE	250/250	63/125	0.25/0.5	0.75/0.75	Additive/ Additive
	Gentamicin	8/16	4/4	0.5/0.25		
S. sanguinis ATCC 10556	PLE	500/1000	125/500	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Gentamicin	8/32	2/8	0.25/0.25		
S. parasanguinis KCOM 1497[4]	PLE	1000/1000	250/500	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Gentamicin	16/32	4/8	0.25/0.25		
S. sobrinus ATCC 27607	PLE	500/1000	125/250	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Gentamicin	8/32	2/8	0.25/0.25		
S. ratti KCTC 3294[5]	PLE	500/1000	125/500	0.25/0.5	0.5/1.0	Synergistic/ Additive
	Gentamicin	8/16	2/8	0.25/0.5		
S. criceti KCTC 3292	PLE	500/2000	125/500	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Gentamicin	16/32	4/8	0.25/0.25		
S. downei KCOM 1165	PLE	1000/1000	500/500	0.5/0.5	0.75/0.75	Additive/ Additive
	Gentamicin	32/64	8/16	0.25/0.25		
S. anginosus ATCC 31412	PLE	1000/2000	250/500	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Gentamicin	8/16	2/4	0.25/0.25		
S. gordonii ATCC 10558	PLE	250/250	63/125	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Gentamicin	16/32	4/8	0.25/0.25		
A. actinomycetemcomitans ATCC 43717	PLE	2000/2000	500/1000	0.25/0.5	0.375/0.75	Synergistic/ Additive
	Gentamicin	16/16	2/4	0.125/0.25		
F. nucleatum ATCC 51190	PLE	500/1000	125/250	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Gentamicin	2/4	0.5/1	0.25/0.25		
P. intermedia ATCC 25611	PLE	1000/2000	250/500	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Gentamicin	32/64	8/16	0.25/0.25		
P. gingivalis ATCC 33277	PLE	250/500	63/125	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Gentamicin	256/512	64/128	0.25/0.25		

[1]The MIC and MBC of 50% ethanol extract of *Paeonia lactiflora* pallas (PLE) with gentamicin
[2] The fractional inhibitory concentration index (FIC index)
[3]American Type Culture Collection (ATCC)
[4]Korean collection for type cultures (KCTC)
Table 3: Synergistic effects of 50% ethanol extract of *Paeonia lactiflora* pallas (PLE) with gentamicin against oral bacteria

Strains	Agent	MIC/MBC (μg/mL)		FIC	FICI[2]	Outcome
		Alone	Combination[1]			
S. mutans ATCC 25175[3]	PLE	250/250	63/125	0.25/0.5	0.5/1.0	Synergistic/ Additive
	Erythromycin	0.063/0.125	0.016/0.063	0.25/0.5		
S. sanguinis ATCC 10556	PLE	500/1000	125/250	0.25/0.25	0.75/0.75	Additive/ Additive
	Erythromycin	0.016/0.031	0.007/0.016	0.5/0.5		
S. parasanguinis KCOM 1497[4]	PLE	1000/1000	250/250	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Erythromycin	0.25/0.5	0.063/0.125	0.25/0.25		

Strains	Agent	MIC/MBC (μg/mL)		FIC	FICI[2]	Outcome
		Alone	Combination[1]			
S. sobrinus ATCC 27607	PLE	500/1000	250/500	0.5/0.5	1.0/0.75	Additive/ Additive
	Erythromycin	0.031/0.063	0.016/0.031	0.5/0.25		
S. ratti KCTC 3294[5]	PLE	500/1000	125/250	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Erythromycin	0.008/0.016	0.002/0.004	0.25/0.25		
S. criceti KCTC 3292	PLE	500/2000	125/250	0.25/0.125	0.5/0.375	Synergistic/ Synergistic
	Erythromycin	0.125/0.25	0.031/0.063	0.25/0.25		
S. downei KCOM 1165	PLE	1000/1000	125/250	0.125/0.25	0.375/0.5	Synergistic/ Synergistic
	Erythromycin	0.25/0.5	0.063/0.125	0.25/0.25		
S. anginosus ATCC 31412	PLE	1000/2000	250/250	0.25/0.125	0.5/0.375	Synergistic/ Synergistic
	Erythromycin	0.25/0.5	0.063/0.125	0.25/0.25		
S. gordonii ATCC 10558	PLE	250/250	63/125	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Erythromycin	0.031/0.063	0.008/0.016	0.25/0.25		
A. actinomycetemcomitans ATCC 43717	PLE	2000/2000	500/500	0.25/0.25	0.375/0.5	Synergistic/ Synergistic
	Erythromycin	0.125/0.25	0.016/0.031	0.125/0.25		
F. nucleatum ATCC 51190	PLE	500/1000	125/250	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Erythromycin	32/64	8/16	0.25/0.25		
P. intermedia ATCC 25611	PLE	1000/2000	250/500	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Erythromycin	16/32	4/8	0.25/0.25		
P. gingivalis ATCC 33277	PLE	250/500	63/63	0.25/0.125	0.75/0.375	Additive/ Synergistic
	Erythromycin	2/8	1/2	0.5/0.25		

[1]The MIC and MBC of 50% ethanol extract of *Paeonia lactiflora* pallas (PLE) with erythromycin
[2]The fractional inhibitory concentration index (FIC index)
[3] merican Type Culture Collection (ATCC)
[4]Korean collection for type cultures (KCTC)
Table 4: Synergistic effects of 50% ethanol extract of *Paeonia lactiflora* pallas (PLE) with erythromycin against oral bacteria

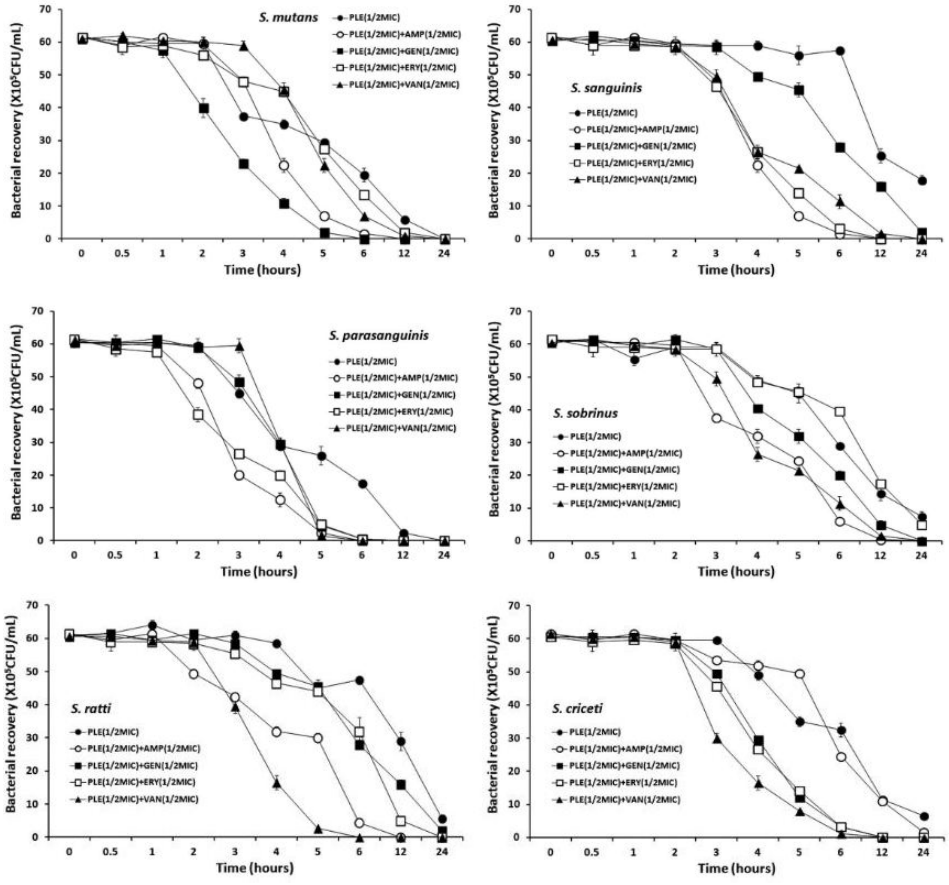
Strains	Agent	MIC/MBC (μg/mL)		FIC	FICI[2]	Outcome
		Alone	Combination[1]			
S. mutans ATCC 25175[3]	PLE	250/250	63/63	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Vancomycin	1/2	0.25/0.5	0.25/0.25		
S. sanguinis ATCC 10556	PLE	500/1000	125/250	0.25/0.25	0.5/0.75	Synergistic/ Additive
	Vancomycin	0.5/1	0.125/0.5	0.25/0.5		
S. parasanguinis KCOM 1497[4]	PLE	1000/1000	250/250	0.25/0.25	0.75/0.75	Additive/ Additive
	Vancomycin	2/4	1/2	0.5/0.5		
S. sobrinus ATCC 27607	PLE	500/1000	125/250	0.25/0.25	0.75/0.5	Additive/ Synergistic
	Vancomycin	1/2	0.5/0.5	0.5/0.25		
S. ratti KCTC 3294[5]	PLE	500/1000	125/250	0.25/0.25	0.5/0.5	Synergistic/ Synergistic
	Vancomycin	1/1	0.25/0.25	0.25/0.25		
S. criceti KCTC 3292	PLE	500/2000	125/500	0.25/0.25	0.75/0.5	Additive/ Synergistic
	Vancomycin	2/4	1/1	0.5/0.25		
S. downei KCOM 1165	PLE	1000/1000	250/250	0.25/0.25	0.5/0.5	Additive/ Synergistic
	Vancomycin	8/16	2/4	0.25/0.25		
S. anginosus ATCC 31412	PLE	1000/2000	250/500	0.25/0.25	0.5/0.375	Synergistic/ Synergistic
	Vancomycin	1/4	0.25/0.5	0.25/0.125		
S. gordonii ATCC 10558	PLE	250/250	63/125	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Vancomycin	0.5/1	0.125/0.25	0.25/0.25		
A. actinomycetemcomitans ATCC 43717	PLE	2000/2000	500/1000	0.25/0.5	0.5/0.75	Synergistic/ Additive
	Vancomycin	2/4	0.5/1	0.25/0.25		
F. nucleatum ATCC 51190	PLE	500/1000	125/250	0.25/0.25	0.375/0.5	Synergistic/ Synergistic
	Vancomycin	64/128	8/32	0.125/0.25		

Strains	Agent	MIC/MBC (μg/mL)		FIC	FICI[2]	Outcome
		Alone	Combination[1]			
P. intermedia ATCC 25611	PLE	1000/2000	250/250	0.25/0.125	0.5/0.375	Synergistic/ Synergistic
	Vancomycin	16/36	4/8	0.25/0.25		
P. gingivalis ATCC 33277	PLE	250/500	63/125	0.25/0.25	0.375/0.5	Synergistic/ Synergistic
	Vancomycin	16/16	2/4	0.125/0.25		

[1]The MIC and MBC of 50% ethanol extract of Paeonia lactiflora pallas (PLE) with vancomycin
[2]The fractional inhibitory concentration index (FIC index)
[3]American Type Culture Collection (ATCC)
[4]Korean collection for type cultures (KCTC)
Table 5: Synergistic effects of 50% ethanol extract of Paeonia lactiflora pallas (PLE) with vancomycin against oral bacteria

The major bioactive components of *P. lactiflora* include monoterpene glycosides, lignans, galloyl glucoses, nortriterpenoids, and phenolic compounds [16,17,22]. The extract of *P. lactiflora* has been demonstrated to exhibit anti-inflammatory, anti-spasmodic, and antiviral activities, in addition to lowering uric acid concentrations [14,15,22,23]. Recently, methyl gallate, paeonol, and 1,2,3,4,6-penta-*O*-galloyl-*β*-d-glucopyranose (PGG) with *P. lactiflora* root extract have been suggested as alternative sources of anti-*Helicobacter pylori* products because of their growth-inhibiting, bactericidal, and urease inhibitory effects [23]. Furthermore, it has been described that phenolic acids can break down the structure of the cytoplasmic membrane causing loss of integrity and eventual cell death [24]. At sub-inhibitory concentrations, the compounds present in the extract would facilitate the entrance of the antibiotic to the cell cytoplasm, thus facilitating the entrance of ampicillin, gentamicin, erythromycin, and vancomycin, which have their site action within the bacterial cell, and less antibiotic dose would be needed. In this way, the multi-objective mechanism would be accomplished by disrupting the cytoplasmic membrane and some vital function as DNA replication, transcription or translation processes, depending on the antibiotic used [24,25].

The bacterial effect of the PLE with ampicillin, gentamicin, erythromycin, and/or vancomycin against oral bacteria was confirmed by time-kill curve experiments. The PLE (MIC or 1/2 MIC) alone resulted rate of killing increasing or not changing in CFU/ml at time-dependent manner, with a more rapid rate of killing by ARE (1/2 MIC) with ampicillin (1/2 MIC), gentamicin (1/2 MIC), erythromycin (1/2 MIC), and/or vancomycin (1/2 MIC) (Figure 1 and 2). The PLE with ampicillin (1/2 MIC) and vancomycin combination was bactericidal effect up to and beyond 12 h exposure with all killing compared to PLE alone in all tested bacteria. The PLE with gentamicin (1/2 MIC) combination was bactericidal effect with all killing up to 12 h exposure in all tested bacteria except *S. sanguinis*, *S. sobrinus*, and *S. ratti*, and with erythromycin except *S. sobrinus* and *S. ratti*.



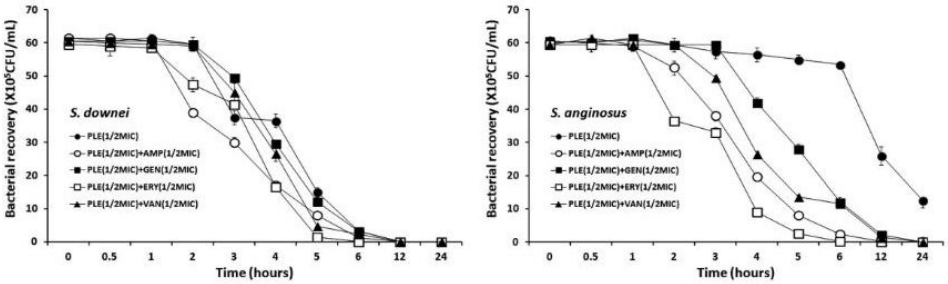


Figure 1: Time-kill curves of MIC of PLE alone and its combination with 1/2 MIC of AMP, GEN, ERY, or/and VAN against *S. mutans, S. sanguinis, S. parasanguinis, S. sobrinus, S. ratti, S. criceti, S. downei*, and *S. anginosus*. Bacteria were incubated with PLE (●), PLE + AMP (○), PLE + GEN (▲), PLE + ERY (△), and PLE + VAN (▪) over time. Data points are the mean values±S.E.M. of six experiments. CFU, colony-forming units

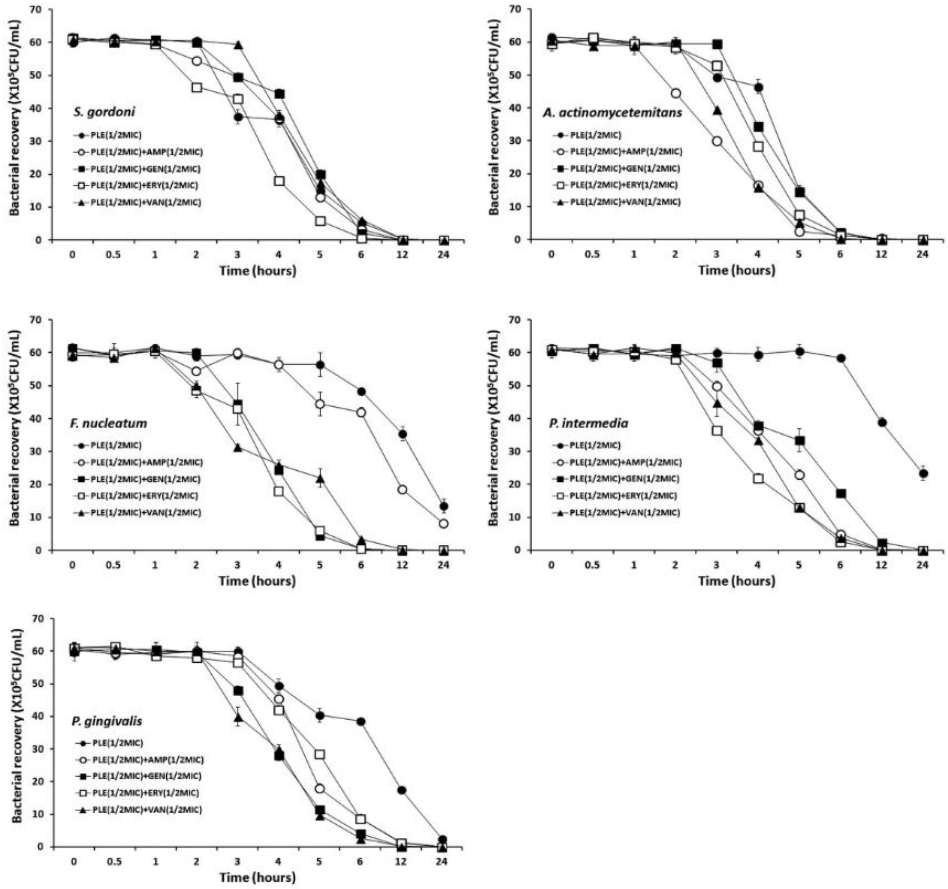


Figure 2: Time-kill curves of MIC of PLE alone and its combination with 1/2 MIC of AMP, GEN, ERY, or/and VAN against *S. gordonii, A. actinomycetemcomitans, F. nucleatum, P. intermedia*, and *P. gingivalis*. Bacteria were incubated with PLE (●), PLE + AMP (○), PLE + GEN (▲), PLE + ERY (△), and PLE + VAN (▪) over time. Data points are the mean values±S.E.M. of six experiments. CFU, colony-forming units

Conclusion

In conclusion, these findings suggest that a strong bactericidal effect of PLE was exerted in drug combinations and fulfills the conditions required of a novel cariogenic bacteria and periodontal pathogens, particularly bacteroides species drug and may be useful in the future in the treatment of oral bacteria.

Competing Interest

The authors declare that they have no competing interests.

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References

1. Patil S, Rao RS, Sanketh DS, Amrutha N (2013) Microbial flora in oral diseases. J Contemp Dent Pract 14: 1202-8.

2. López R, Smith PC, Göstemeyer G, Schwendicke F (2017) Ageing, dental caries and periodontal diseases. J Clin Periodontol 44 Suppl 18: S145-S152.

3. Chi AC, Neville BW, Krayer JW, Gonsalves WC (2010) Oral manifestations of systemic disease. Am Fam Physician 82:1381-8.

4. Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, et al. (2017) Dental caries. Nat Rev Dis Primers 3: 17030.

5. Cardoso EM, Reis C, Manzanares-Céspedes MC (2018) Chronic periodontitis, inflammatory cytokines, and interrelationship with other chronic diseases. Postgrad Med 130: 98-104.

6. Kinane DF, Stathopoulou PG, Papapanou PN (2017) Periodontal diseases. Nat Rev Dis Primers 3: 17038.

7. Aoyama N, Suzuki JI, Kobayashi N, Hanatani T, Ashigaki N, et al. (2018) Associations among tooth loss, systemic inflammation and antibody titers to periodontal pathogens in Japanese patients with cardiovascular disease. J Periodontal Res 53: 117-122.

8. Rowson C, Townsend R (2016) Biofilms: prevention and treatment. Br J Hosp Med 77: 699-703.

9. Levi ME, Eusterman VD (2011) Oral infections and antibiotic therapy. Otolaryngol Clin North Am 44: 57-78.

10. Rams TE, Degener JE, van Winkelhoff AJ (2013) Prevalence of beta-lactamase-producing bacteria in human periodontitis. J Periodontal Res 48: 493-9.

11. Kocsis B, Domokos J, Szabo D (2016) Chemical structure and pharmacokinetics of novel quinolone agents represented by avarofloxacin, delafloxacin, finafloxacin, zabofloxacin and nenonoxacin. Ann Clin Microbiol Antimicrob 15: 34.

12. Mishra BB, Tiwari VK (2011) Natural products: an evolving role in future drug discovery. Eur J Med Chem 46: 4769-807.

13. Zhang W, Dai SM (2012) Mechanisms involved in the therapeutic effects of Paeonia lactiflora Pallas in rheumatoid arthritis. Int Immunopharmacol 14: 27-31.

14. Jiang D, Chen Y, Hou X, Xu J, Mu X, et al. (2011) Influence of Paeonia lactiflora roots extract on cAMP-phosphodiesterase activity and related anti-inflammatory action. J Ethnopharmacol 137: 914-20.

15. Ho JY, Chang HW, Lin CF, Liu CJ, Hsieh CF, et al. (2014) Characterization of the anti-influenza activity of the Chinese herbal plant Paeonia lactiflora. Viruses 6: 1861-75.

16. Li YC, Wang LY, Li R, Wu YZ, Guo QL, et al. (2016) Chemical constituents from ethyl actate exaction of root of Paeonia lactiflora. Zhongguo Zhong Yao Za Zhi 41: 2255-60.

17. Kim JS, Kim YJ, Lee JY, Kang SS (2008) Phytochemical studies on Paeoniae Radix (2)–Phenolic and related compounds. Kor J Pharmacogn 39: 28-36.

18. Abdel-Hafez AA, Meselhy MR, Nakamura N, Hattori M, Watanabe H, et al. (1999) Anticonvulsant activity of paeonimetabolin-I adducts obtained by incubation of paeoniflorin and thiol compounds with Lactobacillus brevis. Biol Pharm Bull 22: 491-7.

19. Adwan G, Abu-Shanab B, Adwan K (2010) Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug-resistant Pseudomonas aeruginosa strains. Asian Pac J Trop Biomed 1: 266-9.

20. Aiyegoro OA, Okoh AI (2009) Use of bioactive plant products in combination with standard antibiotics: Implications in antimicrobial chemotherapy. J Med Plant Res 3: 1147-52.

21. Sanhueza L, Melo R, Montero R, Maisey K, Mendoza L, et al. (2017) Synersistic interactions between phenolic compounds identified in grape pomace extract with antibiotics of different classes against Staphylococcus aureus and Escherichia coli. PLoS One 12: e0172273.

22. Fu Q, Qiu L, Yuan H. Yu M, Zou T, et al. (2016) Paeonenoides D and E: two new nortriterpenoids from Paeonia lactiflora and their inhibitory activities on NO production. Helv Chim Acta 99: 46-9.

23. Ngan LT, Moon JK, Shibamoto T, Ahn YJ (2012) Growth-inhibiting, bactericidal, and urease inhibitory effects of Paeonia lactiflora root constituents and related compounds on antibiotic-susceptible and -resistant strains of Helicobacter pylori. J Agric Food Chem 60: 9062-73.

24. Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12: 564-82.

25. Xie Y, Yang W, Tang F, Chen X, Ren L (2015) Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Curr Med Chem 22: 132-49.