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Antimicrobial Activity of Methanol Extract from *Ficus carica* Leaves Against Oral Bacteria

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Ficus carica L. (fig) belongs to the mulberry tree (*Moraceae*) which is one of the oldest fruits in the world. It has been used as a digestion promoter and a cure for ulcerative inflammation and eruption in Korea. The present study investigated the antimicrobial activity of methanol (MeOH) extract of figs against oral bacteria. The MeOH extract (MICs, 0.156 to 5 mg/ml; MBCs, 0.313 to 5 mg/ml) showed a strong antibacterial activity against oral bacteria. The combination effects of MeOH extract with ampicillin or gentamicin were synergistic against oral bacteria. We suggest that figs could be employed as a natural antibacterial agent in oral care products.

Key Words: *Ficus carica* (fig), Antibacterial activity, MIC/MBC, Synergistic

INTRODUCTION

Ficus carica Linn. (Syn: *Ficus sycomorous*; family: *Moraceae*) is commonly referred as ""Fig". The cultivated Fig, native to the arid region of Asia Minor, forms a shrub or low-spreading deciduous tree. The large, wavy-margined leaves are usually 5 lobed but may have only 4 or 3 lobes (1). Its fruit, root and leaves are used in the native system of medicine in various disorders such as gastrointestinal (colic, indigestion, loss of appetite and diarrhea), respiratory (sore throats, coughs and bronchial problems), inflammatory, cardiovascular disorders, ulcerative diseases, and cancers (1~5). *F. carica* has been reported to have numerous bioactive compounds such as arabinose, β-amyrins, β-carotines, glycosides, β-setosterols and xanthotoxol (5~7). The researchers reported the hypoglycemic action of a fig leaf decoction in type-I diabetic patients and used a chloroform extract, obtained also from a decoction of *F. carica* leaves, to decrease the cholesterol levels of rats with diabetes (8). *F. carica* has been reported to include antioxidant, antiviral, antibacterial, hypoglycemic, hypocholesterolaemic, cancer suppressive, hypotriglyceridaemic, and anthelmintic effects (2~4, 9~11). It has also been investigated for its proteolytic enzymes, amino acids, minerals, sugars, triterpenes, organic acids, and allergens (1, 6).

This study was aimed at providing the antimicrobial activities of *F. carica* (figs) MeOH extract against oral bacteria.

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MATERIAL AND METHODS

Plant material and preparation of methanol extract

F. carica leaves were collected in September 2005 from the Samho farm of Yeongam-gun in Korea. The identity was confirmed by Dr. Bong-Seop Kil, College of Natural Science, Wonkwang University. The voucher specimens

(DJ-05-F1) were deposited at the Herbarium of the College of Natural Science, Wonkwang University. The dried and powered leaves (1.2 kg) of *F. carica* were extracted by repeated refluxing with methanol (MeOH) (2 × 6 L) for 4 h at 80℃. The combined MeOH extract (12 L) was clarified by filtration and evaporated to obtain dark green syrup (210 g).

Minimum inhibitory concentration/minimum bactericidal concentration assay

The antimicrobial activity of the MeOH extract of *F. carica* leaves against oral bacteria: *Streptococcus mutans* (ATCC 25175), *Streptococcus sanguinis* (ATCC 10556), *Streptococcus sobrinus* (ATCC 27607), *Streptococcus ratti* (KCTC 3294), *Streptococcus criceti* (KCTC 3292), *Streptococcus anginosus* (ATCC 31412) and *Streptococcus gordonii* (ATCC 10558), *Aggregatibacter actinomycetemcomitans* (ATCC 43717), *Fusobacterium nucleatum* (ATCC 51190), *Prevotella intermedia* (ATCC 49046), and *Porphyromonas gingivalis* (ATCC 33277) was determined through the broth dilution method carried out in triplicate. The reference strains used in this study were: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, and *Streptococcus pyogenes* ATCC 21059.

The minimum inhibitory concentration (MIC) was determined as the lowest concentration of test samples that resulted in a complete inhibition of visible growth in the broth. Following anaerobic incubation of MIC plates, the minimum bactericidal concentration (MBC) was determined on the basis of the lowest concentration of the MeOH extract that kill 99.9% of the test bacteria by plating out onto each appropriate agar plate.

Checker board dilution test

The synergistic effects of the MeOH extract, which exhibited the highest antimicrobial activity and antibiotics, were assessed by the checkerboard test as previously described (12). The antimicrobial combinations assayed included the MeOH extract plus ampicillin or gentamicin. The (FICI) is the sum of the FICs of each of the drugs, which in turn is defined as the MIC of each drug when it is used in combination divided by the MIC of the drug when it is used alone. The interaction was defined as synergistic if the FICI was less than or equal to 0.5, additive if the FICI was greater than 0.5 and less than or equal 1.0, indifferent if the FICI was greater than 1.0 and less than or equal to 2.0, and antagonistic if the FICI was greater than 2.0 (12).

RESULTS

The results of the antibacterial activity showed that the MeOH extract of *F. carica* leaves exhibited strong activities against *S. gordonii*, *S. anginosus*, *P. intermedia*, *A. actinomycetemcomitans*, and *P. gingivalis* (MIC, 0.156 to 0.625 mg/ml; MBC, 0.313 to 0.625 mg/ml), and moderate antibacterial activity against the other bacteria (MIC, 1.25 mg/ml; MBC, 1.25 to 2.5 mg/ml), while *E. coli*, *S. aureus*, *S. sanguinis*, and *S. criceti* appeared to be less sensitive (MIC, 2.5 to 10 mg/ml; MBC, 2.5 to 10 mg/ml). The MIC and MBC for ampicillin were found to be either 0.5/0.5 or 256/256 μg/ml; for gentamicin, either 2/2 or 256/512 μg/ml (Table 1, 2).

The combination effects of MeOH extract with ampicillin or gentamicin against oral bacteria and a few reference strains were presented in Tables 1, 2. In combination with MeOH extract, the MIC for ampicillin was reduced ≥4-8-fold and MeOH extract indicated ≥2-8-fold in most of tested bacteria, producing a synergistic effect as defined by FICI ≤ 0.375~0.5. The additive effect of MeOH extract with ampicillin combination led to a reduction of a single or double dilution in *E. coli*, *S. ratti*, and *F. nucleatum*, and as defined by FICI ≤ 0.75 (Table 1).

The combination of the MeOH extract with gentamicin resulted in the decrease in MIC for all tested bacteria (≥2-8-fold), with the MIC of 0.5~32 μg/ml for gentamicin becoming 2~256 μg/ml and MIC of 0.039~2.5 mg/ml for the MeOH extract becoming 0.156~10 mg/ml. The FICI classified the combination of the MeOH extract with gentamicin as a synergistic effect (FICI ≤ 0.375~0.5) for all tested bacteria except an additive effect, such as *S. pyogenes*, *S. sanguinis*, *S. criceti*, *P. intermedia*, and *F. nucleatum* (Table 2).

Table 1. Checkerboard assay of the MeOH extract of *F. carica* leaves and ampicillin for some oral bacteria with a few reference strains

Strains	Agent	MIC/MBC[a]		FIC[c]	FICI[c]	Outcome
		Alone	Combination[b]			
E. coli ATCC 25922	MeOH	10/10	5/5	0.5	0.75	Additive
	Ampicillin	256/256	64/64	0.25		
S. aureus ATCC 29213	MeOH	2.5/2.5	0.625/0.625	0.25	0.5	Synergistic
	Ampicillin	16/16	4/8	0.25		
S. epidermidis ATCC 12228	MeOH	1.25/1.25	0.313/0.625	0.25	0.5	Synergistic
	Ampicillin	32/64	8/16	0.25		
S. pyogenes ATCC 21059	MeOH	1.25/2.5	0.313/0.625	0.25	0.5	Synergistic
	Ampicillin	4/8	1/1	0.25		
S. mutans ATCC 25175	MeOH	1.25/1.25	0.313/0.313	0.25	0.5	Synergistic
	Ampicillin	0.5/0.5	0.125/0.125	0.25		
S. sanguinis ATCC 10556	MeOH	2.5/2.5	0.313/0.625	0.125	0.375	Synergistic
	Ampicillin	1/4	0.25/0.5	0.25		
S. sobrinus ATCC 27607	MeOH	1.25/2.5	0.313/0.313	0.25	0.375	Synergistic
	Ampicillin	0.125/0.5	0.0156/0.0312	0.125		
S. ratti KCTC 3294	MeOH	1.25/2.5	0.625/0.625	0.5	0.75	Additive
	Ampicillin	1/2	0.25/0.5	0.25		
S. criceti KCTC 3292	MeOH	2.5/5.0	0.625/0.625	0.25	0.5	Synergistic
	Ampicillin	1/1	0.25/0.5	0.25		
S. anginosus ATCC 31412	MeOH	0.625/0.625	0.156/0.156	0.25	0.5	Synergistic
	Ampicillin	0.5/1	0.125/0.25	0.25		
S. gordonii ATCC 10558	MeOH	0.156/0.313	0.039/0.078	0.25	0.5	Synergistic
	Ampicillin	1/1	0.25/0.25	0.25		
A. actinomycetemcomitans ATCC 43717	MeOH	0.625/0.625	0.156/0.156	0.25	0.5	Synergistic
	Ampicillin	32/32	8/16	0.25		
F. nucleatum ATCC 51190	MeOH	1.25/1.25	0.0.625/0.625	0.5	0.75	Additive
	Ampicillin	2/4	0.5/1	0.25		
P. intermedia ATCC 49049	MeOH	0.313/0.313	0.078/0.156	0.25	0.5	Synergistic
	Ampicillin	8/8	2/2	0.25		
P. gingivalis ATCC 33277	MeOH	0.625/0.625	0.156/0.156	0.25	0.5	Synergistic
	Ampicillin	0.5/1	0.125/0.125	0.25		

[a] The MeOH extract: mg/ml, ampicillin: μg/ml

[b] The checkerboard test was performed as previously described (12). The MICs and MBCs of the MeOH extract with ampicillin against oral bacteria are indicated.

[c] The interaction was defined as synergistic if the FICI was less than or equal to 0.5, additive if the FICI was greater than 0.5 and less than or equal 1.0, indifferent if the FICI was greater than 1.0 and less than or equal to 2.0, and antagonistic if the FICI was greater than 2.0 (12)

Table 2. Checkerboard assay of the MeOH extract of *F. carica* leaves and gentamicin for some oral bacteria with a few reference strains

Strains	Agent	MIC/MBC[a]		FIC[c]	FICI[c]	Outcome
		Alone	Combination[b]			
Escherichia coli ATCC 25922	MeOH	10/10	2.5/2.5	0.25	0.5	Synergistic
	Gentamicin	16/16	4/8	0.25		
Staphylococcus aureus ATCC 29213	MeOH	2.5/2.5	0.625/1.25	0.25	0.5	Synergistic
	Gentamicin	2/2	0.5/0.5	0.25		
Staphylococcus epidermidis ATCC 12228	MeOH	1.25/1.25	0.313/0.625	0.25	0.5	Synergistic
	Gentamicin	2/2	0.5/1	0.25		
Streptococcus pyogenes ATCC 21059	MeOH	1.25/2.5	0.625/0.625	0.5	0.75	Additive
	Gentamicin	8/16	2/2	0.25		
S. mutans ATCC 25175	MeOH	1.25/1.25	0.313/0.313	0.25	0.5	Synergistic
	Gentamicin	8/16	2/4	0.25		
S. sanguinis ATCC 10556	MeOH	2.5/2.5	0.625/1.25	0.25	0.75	Additive
	Gentamicin	32/64	16/16	0.5		
S. sobrinus ATCC 27607	MeOH	1.25/2.5	0.313/0.625	0.25	0.5	Synergistic
	Gentamicin	8/8	2/2	0.25		
S. ratti KCTC 3294	MeOH	1.25/2.5	0.313/0.625	0.25	0.5	Synergistic
	Gentamicin	16/32	4/8	0.25		
S. criceti KCTC 3292	MeOH	2.5/5.0	0.625/1.25	0.25	0.75	Additive
	Gentamicin	8/8	4/4	0.5		
S. anginosus ATCC 31412	MeOH	0.625/0.625	0.156/0.156	0.25	0.5	Synergistic
	Gentamicin	32/64	8/16	0.25		
S. gordonii ATCC 10558	MeOH	0.156/0.313	0.039/0.078	0.25	0.5	Synergistic
	Gentamicin	32/64	8/16	0.25		
A. actinomycetemcomitans ATCC 43717	MeOH	0.625/0.625	0.156/0.156	0.25	0.5	Synergistic
	Gentamicin	8/8	2/4	0.25		
F. nucleatum ATCC 51190	MeOH	1.25/1.25	0.313/0.313	0.25	0.75	Additive
	Gentamicin	4/4	2/2	0.5		
P. intermedia ATCC 25611	MeOH	0.313/0.313	0.078/0.078	0.25	0.75	Additive
	Gentamicin	16/32	8/8	0.5		
P. gingivalis ATCC 33277	MeOH	0.625/0.625	0.156/0.156	0.25	0.375	Synergistic
	Gentamicin	256/512	32/64	0.125		

[a] The MeOH extract: mg/ml, gentamicin: μg/ml

[b] The checkerboard test was performed as previously described (12). The MICs and MBCs of the MeOH extract with gentamicin against oral bacteria are indicated.

[c] The interaction was defined as synergistic if the FICI was less than or equal to 0.5, additive if the FICI was greater than 0.5 and less than or equal 1.0, indifferent if the FICI was greater than 1.0 and less than or equal to 2.0, and antagonistic if the FICI was greater than 2.0 (12)

DISCUSSION

The phytochemical analysis reveals that the aqueous extract of ripe dried fruit of *F. carica* contains alkaloids, flavonoids, coumarins, saponins, and terpenes (6, 13). Some phenolic compounds, with reported pharmacological properties have already been isolated from fig leaves, namely furanocoumarins like psoralen and bergapten, flavonoids like rutin, quercetin, and luteolin, phenolic acids like ferrulic acid, and also phytosterols like taraxasterol (6, 7). Phenolic compounds constitute an important class of phytochemicals which possess diverse biological activities like astringent, antioxidant, anticancer, anti-inflammation, and antibacterial activity, etc (2, 6, 14). In this study, the antibacterial activity of the MeOH extract of *F. carica* leaves showed strong activities against *S. gordonii*, *S. anginosus*, *P. intermedia*, *A. actinomycetemcomitans*, and *P. gingivalis* (MIC, 0.156 to 0.625 mg/ml; MBC, 0.313 to 0.625 mg/ml). Some phenolic compounds isolated from plants exhibit anticaries activity either due to growth inhibition against mutans streptococci or due to the inhibition of glucosyltransferases (15). As several reports have demonstrated that some flavonoid compounds evidence antibacterial activity against oral bacteria (16), it is generally considered that the flavonoids in *F. carica* may be related, in part, to the antibacterial effects observed in the present study. The synergistic effects of the MeOH extract with ampicillin or gentamicin combination against oral bacteria were presented as ≥4-8-fold reduction of MIC, producing a synergistic effect as defined by FICI ≤ 0.375~0.5. Especially, MeOH extract with ampicillin or gentamicin combination showed the strongest synergistic effect (FICI ≤ 0.375) against *S. sanguinis*, *S. sobrinus*, and *P. gingivalis*.

The water extract and ethyl acetate and hexane fractions from methanol extracts from the leaves of *F. carica* have been demonstrated as anti-HSV-1 effect and insecticidal activity against *Tetranychus urticae* (9, 17). The administration of the basic and chloroform extracts of *F. carica* affect the oxidative stress in diabetes, with particular significance regarding the vitamin E/C18:2 ratio when the chloroform fraction is administered, and the vitamin A/C18:2 ratio with the basic fraction (8). The 6-*O*-acyl-β-d-glucosyl-β-sitosterols along with its palmitoyl, linoleyl, stearyl and oleyl derivatives isolated from the fruit of *F. carica* exhibited strong cytotoxic effect (2). The *F. carica* leaves are composed of many flavonoids then it may perhaps indicate strong antibacterial activity against oral bacteria.

These findings suggest that a strong bactericidal effect was exerted in drug combinations. The *F. carica* leaves fulfill the conditions required for a novel agent against cariogenic bacteria and periodontal pathogens and could be employed as a natural antibacterial agent in oral care products.

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