Antimicrobial Activity of Seeds of *Zanthoxylum piperitum* against Oral Pathogen *Streptococcus mutans*

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Antimicrobial activity of *Zanthoxylum piperitum* was investigated against *Streptococcus mutans* that causes dental caries. Although the methylene chloride extract of seeds exhibited higher antimicrobial activity than other organic solvent extracts, including methanol, ethyl acetate, and hexane extracts of pericarps or seeds of *Z. piperitum*, essential oils prepared from both seeds and pericarps possessed more potent inhibitory activity than the methylene chloride extract of seeds. The minimal inhibitory concentrations (MICs) of the essential oils of seeds and pericarps were 0.3 mg/ml and 4.0 mg/ml against *S. mutans*, respectively. When the seed essential oil was further separated into seven fractions (CS-SD-A~CS-SD-G) by thin layer chromatography (TLC), all fractions exhibited lower antimicrobial activity than the essential oil. To understand the antimicrobial ingredients of *Z. piperitum*, seeds the gas chromatography-mass spectrometry (GC-MS) data of the methylene chloride extract of seeds was compared with those of the seed essential oil (CS-SD). Whereas the methylene chloride extract of seeds contained carvacrol (0.24%), β-caryophyllene (1.72%), and α-humulene (0.88%), which were previously known to inhibit growth of *S. mutans*, the seed essential oil contained sabinene (1.57%), linalool (1.55%), citronellal (13.67%), terpinene-4-ol (0.45%), citronellol (3.69%), geraniol (0.9%), linalyl acetate (1.35%), β-caryophyllene (1.35%), α-humulene (0.78%), and δ-cadinene (0.67%) in this regard. These results indicate that *Z. piperitum* seeds possess various inhibitory substances against *S. mutans*, and an effective method to isolate the active ingredients from the seeds is to prepare the essential oil. These results also suggest that the essential oil of *Z. piperitium* seeds may be applicable to preventing dental caries.

Key words : *Zanthoxylum piperitum*, antimicrobial activity, essential oil, *Streptococcus mutans*, minimum inhibitory concentration (MIC), gas chromatography-mass spectrometry (GC-MS)

Introduction

Streptococcus mutans is known to be one of the causative agents of dental caries, which leads to tooth dysfunction, periodontal lesions, and tooth loss [14,26]. Since *S. mutans* produces the glucosyltransferase that forms a sticky polysaccharide dextran from sucrose, the growth of *S. mutans* can mediate adherence of itself and other acid-forming bacteria on tooth surfaces as a thick layer called dental plaque [31]. Sequentially, the acid-forming bacteria including *Lactobacillus* sp, lower pH levels by secreting lactic acid and thus increase the probability of decalcification of the tooth enamel, which finally results in dental caries [1,28].

Many attempts have been made to eliminate *S. mutans* from the oral microflora. Antibiotics including ampicillin, chlorhexidine, erythromycin, penicillin, tetracycline and vancomycin, which control the bacterial growth, have been very effective in preventing dental caries [19]. However, excessive use of these antibiotic drugs can cause a significant alteration of the oral and intestinal microflora and thus provokes undesirable side effects including enhanced susceptibility to pathogenic microorganisms, vomiting, and diarrhea [9]. Natural products have recently been demonstrated as an alternative to those antibiotics for the prevention of dental caries [21,22]. Essential oils are among these important natural products. Although essential oils have a long history as traditional medicinal agents and are known to possess several biological functions, including anticancer, antiviral, antimutagenic and anti-inflammatory activities [2,29], some essential oils are evaluated as ideal antibacterial agents, which can exert inhibitory effect on a broad range of microorganisms, maintain activity at a low concentration, and are applicable without side effects. In this context, several plant derivatives of *Lippia sidoides* (verbenaceae), *Myristica fragrans* (nutmeg), *Cinnamomum verum* (cinnamon), *Melaleuca alter-*

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nifolia (tea-tree), *Leptospermum scoparium* (manuka), *Leptospermum morrisonii* (large-leaf yellow tea-tree), *Arnica montana* (arnica), *Eucalyptus radiata* (eucalyptus), *Citrus paradisi* (grapefruit) etc. have been evaluated for their antimicrobial activities against *S. mutans* [9,13,27].

Fruits and other parts from an aromatic plant *Zanthoxylum piperitum* and other species of *Zanthoxylum* have been widely used for as a pungent condiment and seasoning in north-eastern countries such as Korea and Japan [4,8]. In addition, they have been employed as traditional herbal medicine for treatment of vomiting, diarrhea, and abdominal pain [17]. It is of interest that nonvolatile and volatile oils prepared from fruits of *Z. piperitum* could reduce food consumption in rodents possibly due to causing very unpleasant and long lasing oral sensation [11,12]. The fruits of a plant belonging to *Zanthoxylum* genus have been reported to exert analgesic effects on toothache [18]. In several studies, the composition of fruits of *Z. piperitum* has been analyzed by the gas chromatography-mass spectrometry (GC-MS), and it appears to be chemically complex and many of the ingredients are volatile. It has been reported that the fruit of *Z. piperitum* contains limonene, α-terpineol, linalool, citral, citronellal, cineol dipentene, and geraniol as the major components [4,15,20]. However, the inhibitory activity of organic solvent extracts as well as essential oils prepared from *Zanthoxylum piperitum* against *S. mutans* has been poorly understood.

In the present study, we have examined whether *Z. piperitum* can inhibit the growth of *S. mutans* and have compared antimicrobial activities of methanol, methylene chloride, ethyl acetate, and hexane extracts of pericarps or seeds with those of essential oils prepared from pericaps or seeds of *Z. piperitum* in order to determine the effective method to isolate the antimicrobial ingredients from *Z. piperitum*. In addition, to obtain an insight into the active ingredient (s) responsible for antimicrobial activity, we analyzed the chemical composition of methylene chloride extract as well as essential oil of seed of *Z. piperitum* by GC-MS.

Materials and Methods

Microbial strain and medium

To determine antimicrobial activity of organic solvent extracts or essential oils of *Zanthoxylum piperitum*, a bacterial strain *Streptococcus mutans* (ATCC 25175) were used. For the cultivation of *S. mutans*, the Brain Heart Infusion (BHI) broth was purchased from Becton Dickinson (Sparks, MD, USA). To obtain exponentially growing culture of *S. mutans*, the cells grown in the BHI broth at 37°C overnight were inoculated into 10 ml of the BHI broth and were incubated at 37°C for 3 hr.

Organic solvent extraction from pericarps or seeds

Zanthoxylum piperitum were collected from GyeongSan, Gyeongsangbuk-do, Korea and dried at room temperature. The extract from each portions of *Zanthoxylum piperitum* prepared and a voucher specimen of pericarps, seeds is preserved at the Department of Pharmacy, Catholic University of Daegu, Korea. The dried pericarp of *Z. piperitum* (10 g) were powdered and exhaustively extracted with 100 ml of methanol at 25°C for 10 min. This procedure was repeated three times. The ethyl acetate, hexane and methylene chloride extracts of pericarps were obtained by the same procedure used for the methanol extract. The extracts of seed (10 g) were prepared by using following method, which was extracted with 100 ml of each solvent at 30°C for 10 min, three times.

Preparation of essential oils and TLC fractionation

To extract essential oils, either seeds or pericarps of *Z. piperitum* were subjected to hydrodistillation using a distillation apparatus. Briefly, seed (30 g) or pericarps (30 g) mixed with 300 ml of distilled water were added to a distillation apparatus, and then extracxtion was carried out at 130~150°C for 5 hr. To further fractionation of the essential oil, the essential oil (1 g) dissolved in 2 ml of methylene chloride were resolved by TLC on a silica gel (No. 1.13895, silicagel F_{254} 1 mm, 20×20 cm) using hexane-ethyl acetate (10:1) as the solvent system. The seven fractions were visualized under UV light (UV-160A) at 254 nm and were eluted with methanol.

Antimicrobial activity assay

The disc diffusion method was employed for the evaluation of antimicrobial activity. Briefly, 15 μl of exponentially growing culture of *S. mutans* was mixed with 3 ml of 0.7% BHI agar medium were spread over 1.5% BHI agar plate. The discs (7 mm in diameter) were impregnated with 30 μl of sample solution dissolved in dimethyl sulfoxide (DMSO) at concnentrations of 33.3 mg/ml, 66.7 mg/ml, or 100 mg/ml, and then placed onto the inoculated agar plate. The inoculated agar plates were incubated at 37°C for 24

hr. Antimicrobial activity was evaluated by measuring the diameter of inhibition zone against *Streptococcus mutans*. In order to determine the minimal inhibitory concentration (MIC), 20 μl of exponentially growing culture of *S. mutans* was inoculated into 20 ml of BHI broth containing various concentrations of samples, and then cultured with shaking at 37°C for 24 hr. Bacterial growth was measured using spectrophotometer at 600 nm and the lowest concentration of sample which could inhibit a visible growth of bacteria was determined as the MIC [3].

Analysis of mehtylene chloride extract and essential oil by gas chromatography-mass spectrometry (GC-MS)

The gas chromatography-mass spectrometry (GC-MS) analysis was conducted with Hewlett-Packard (HP) 6890 gas chromatograph coupled to an HP5973N mass spectrometer. A HP-5MS capillary column filled with 5% Phenyl Methyl Siloxane was connected to the GC instrument. The GC analytical conditions were as follows: helium carrier gas flow rate, 0.7 ml/min; oven temperature program, 60°C (hold for 15 min) rising to 280°C; and split ratio, 30:1. The MS instrument was operated in the electron impact (EI) mode and scanned at 70 eV in an *m/z* range of 50-800 mass unit.

Results and Discussion

Antimicrobial activity of organic solvent extracts of *Zanthoxylum piperitum* against *Streptococcus mutans*

In many studies, natural products of plant origin appeared to possess antimicrobial activity against an oral pathogen *Streptococcus mutans* [16,21,27]. In order to investigate an inhibitory activity of *Zanthoxylum piperitum* against *S. mutans*, seeds and other parts including pericarps, roots, stems, and leaves of the plant were extracted with various organic solvents such as methanol, methylene chloride, ethyl acetate, and hexane, and then the individual extractions were tested for antimicrobial activity by the disc diffusion method at concentrations of 10, 20, or 30 mg/disc. When diameters of inhibition zone of the individual extracts against the growth of *S. mutans* were compared, the organic solvent extracts of pericarps, roots, stems, or leaves exhibited no inhibition toward growth of *S. mutans* (Table 1). Although the methanol extract, ethyl acetate extract, or hexane extract of seeds showed antimicrobial activity against *S. mutans* at a concentration of 3 mg/disc with the inhibition zones ranging from 11.6 to 15.6 mm, they failed to inhibit the bacterial growth at a concentration of 2 mg/disc. However, the methylene chloride extract of seeds was able to inhibit the growth of *S. mutans* even at concentrations of 2 mg/disc and 3 mg/disc, but was unable to suppress the bacterial growth at a concentration of 1 mg/disc. While the inhibition zone for the concentration of 3 mg/disc was 16.4 mm, it was 8.0 mm for the 2 mg/disc concentration. These results indicate that seeds of *Z. piperitum* have antimicrobial activity against *S. mutans*, and suggest that the extraction of seeds with methylene chloride might be an effective method to isolate the antimicrobial ingredient(s) from the seeds.

Table 1. Antimicrobial activity of the organic solvent extracts from *Zanthoxylum piperitum* against *S. mutans*

Extract	Zone of inhibition (mm)		
	1	2	3 (mg/disc)
CR[a]-methanol	-	-	-
CR-ethyl acetate	-	-	-
CR-hexane	-	-	-
CR-methylene chloride	-	-	-
CST[b]-methanol	-	-	-
CST-ethyl acetate	-	-	-
CST-hexane	-	-	-
CST-methylene chloride	-	-	-
CL[c]-methanol	-	-	-
CL-ethyl acetate	-	-	-
CL-hexane	-	-	-
CL-methylene chloride	-	-	-
CP[d]-methanol	-	-	-
CP-ethyl acetate	-	-	-
CP-hexane	-	-	-
CP-methylene chloride	-	-	-
CS[e]-methanol	-	-	11.6
CS-ethyl acetate	-	-	13.2
CS-hexane	-	-	15.6
CS-methylene chloride	-	8.0	16.4

[a]Root of *Zanthoxylum piperitum*.
[b]Stem of *Zanthoxylum piperitum*.
[c]Leave of *Zanthoxylum piperitum*.
[d]Pericarp of *Zanthoxylum piperitum*.
[e]Seed of *Zanthoxylum piperitum*.

Inhibitory effect of essential oils prepared from pericarps or seeds of Z. *piperitum* on growth of S. *mutans*

Since the antimicrobial activity assay for the organic solvent extracts of various parts of *Z. piperitum* revealed that

the presence of antimicrobial activity in organic solvent extracts of *Z. piperitum* seeds with a maximum activity in the methylene chloride extract of seeds, we decided to examine the antimicrobial activity of essential oils of pericarps and seeds of *Z. piperitum*, which were prepared by steam distillation. When the inhibitory activity of the essential oils against *S. mutans* was measured by a disc diffusion method, the inhibition zones for the seed essential oil and the pericarp essential oil at a concentration of 3 mg/disc appeared to be 20.0 mm and 25.6 mm, respectively. This indicated that the antimicrobial activities of both essential oils were more potent than that of the methylene chloride extract of seeds of *Z. piperitum* (Table 2). To compare further the antimicrobial activity between the seed essential oil and the pericarp essential oil, the minimal inhibitory concentrations (MICs) of both essential oils against growth of *S. mutans* in the BHI broth were investigated. As shown in Fig. 1 and 2, the MICs of the seed essential oil and pericarp essential oil were 0.3 mg/ml and 4 mg/ml, respectively, demonstrating that the inhibitory activity of the seed essential oil was significantly higher than that of the pericarp essential oil.

Table 2. Antimicrobial activity of seed, pericarp oil from *Zanthoxylum piperitum* against *S. mutans*

Extract	Zone of inhibition (mm)		
	1	2	3 (mg/disc)
CP-SD[a]	-	-	20.0
CS-SD[b]	-	9.5	23.6

[a]Pericarp oil of *Zanthoxylum piperitum* by steam distillation.
[b]Seed oil of *Zanthoxylum piperitum* by steam distillation.

Previously, it was reported that the MIC value of an ethanol extract of leaves of *Streblus asper* against *S. mutans* was 1.93±0.08 mg/ml [30]. It was also reported that the essential oil prepared from *Dracocephalum foetidum* could exert a strong inhibition against *S. mutans* at a concentration of 1.035 mg/ml [25]. It is noteworthy that, in the literature, the MIC values for 1,8-cineol, menthol, and thymol, which are known to comprise the commercial mouthwash products for preventing dental caries, appeared to be higher than the MIC value of the seed essential oil of *Z. piperitum* [9,32]. Recently Cha et al. have reported that the MIC values of the essential oils of *Cryptomeria japonica* and *Artemisia feddei* against *S. mutans* are 0.1 mg/ml and 0.4 mg/ml, respectively [6,7]. It has also been shown that the MIC value of the essential oil prepared from *Origanum majorana* L., which can be applicable as antimicrobial agent against *S. mutans* in sausage, is 2.3 mg/ml [5]. These previous and current results suggest that the essential oil of *Z. piperitum* seeds might be applicable to controlling *S. mutans*.

To fractionate further the antimicrobial components contained in the seed essential oil, the essential oil was separated into seven fractions (CS-SD-A~CS-SD-G) by thin layer chromatography (TLC). When the antimicrobial activity of the individual fractions against *S. mutans* were measured by the disc diffusion method, the fifth fraction CS-SD-E exhibited the strongest inhibitory activity with the inhibition zone of 18.9 mm at a concentration of 3 mg/disc (Table 3). All fractions including CS-SD-E fraction, however, showed less antimicrobial activity than did the seed essential oil. Consequently, these results suggest that the

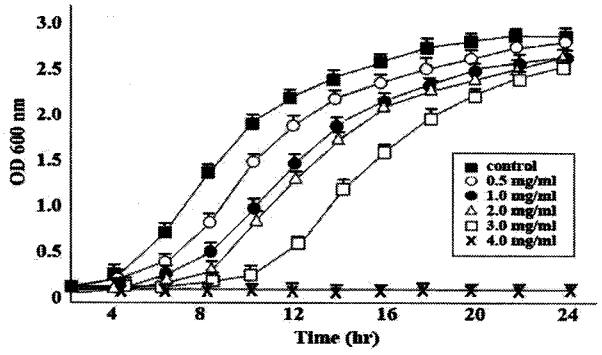

Fig. 1. Inhibitory effect of the essential oils of *Zanthoxylum piperitum* pericarps on the growth of *Streptococcus mutans*. Inhibitory effect of the pericarp essential oil against *S. mutans* was evaluated at various concentrations ranging from 0 to 4 mg/ml for 24 hr.

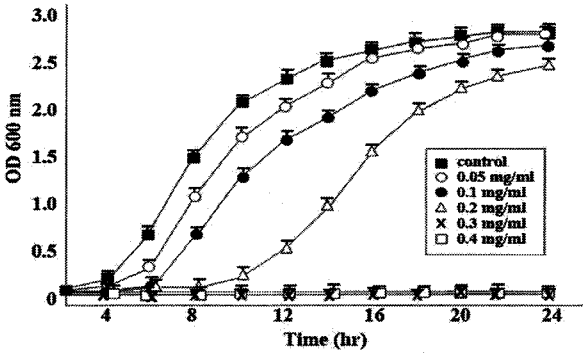

Fig. 2. Inhibitory effect of the essential oils of *Zanthoxylum piperitum* seeds on the growth of *Streptococcus mutans*. Inhibitory effect of the seed essential oil against *S. mutans* was evaluated with various concentrations ranging from 0 to 0.4 mg/ml for 24 hr.

Table 3. Antimicrobial activity of TLC fractions isolated from *Zanthoxylum piperitum* against *S. mutans*

Extract	Zone of inhibition (mm)
	3 (mg/disc)
CS-SD[a]-A	10.5
CS-SD-B	13.8
CS-SD-C	-
CS-SD-D	12.6
CS-SD-E	18.9
CS-SD-F	15.0
CS-SD-G	9.0
CS-SD	24.4

[a]Seed oil of *Zanthoxylum piperitum* by steam distillation.

inhibitory effect of the seed essential oil on *S. mutans* might be attributable to a mixture of active ingredients but not to a single component.

Comparison of the chemical composition of the methylene chloride extract of seeds with that of the essential oil of seeds by GC-MS analysis

To identify the active ingredient (s) in seeds of *Z. piperitum*, the methylene chloride extract (CS-methylene chloride) and essential oil of seeds, both of which appeared to possess antimicrobial activity, were dissolved in DMSO and analyzed by the GC-MS. As shown in Table 4, a total of twenty compounds were identified in the CS-methylene chloride, representing 98.59% of the total composition. The main components were neryl acetate (28.79%), geranyl acetate (22.5%), and citronella (22.09%). Geranyl acetate was previously detected as one of the main components of the essential oil from *Cymbopogon martini* and was shown to inhibit *Escherichia coli* with the MIC value of 0.5 mg/ml [10]. Among the minor components, carvacrol (0.24%), β-caryophyllene (1.72%), and α-humulene (0.88%) were reported to possess the inhibitory activity against *S. mutans* with the MIC values of 2.5 mg/ml [3], 1.6 mg/ml [7], and 0.8 mg/ml [23], respectively. On the other hand, GC-MS analysis revealed that the seed essential oil contained a total of forty-four components (Table. 5). As the major components, γ-terpinene (21.41%), citronellal (13.67%), lavandulyl acetate (12.44%), 1-β-pinene (8.40%) and citronellyl acetate (5.60%) were detected. In addition, various minor components were also detected including sabinene (1.57%), linalool (1.55%), citronellal (13.67%), terpinene-4-ol (0.45%), citronellol (3.69%), geraniol (0.9%), linalyl acetate (1.35%), β-caryophyllene (1.35%), α-humulene (0.78%), and δ-cadinene (0.67%) which

Table 4. The chemical composition of CS-methylene chloride extract from *Zanthoxylum piperitum* seed

Peak no.[a]	Compound	RI[b]	Peak area (%)[c]
1	geranyl formate	6.93	2.90
2	neryl acetate	7.48	28.79
3	linalyl formate	8.83	0.46
4	citronella	9.03	22.09
5	*p*-menth-2-en-1-ol	9.44	0.14
6	Isopulegol 3	9.74	0.42
7	cryptone	9.94	2.24
8	cyclopentane, (2-methylpropylidene)-	10.13	0.13
9	β-citronellol	10.69	2.04
10	linalyl acetate	11.11	1.06
11	carvacrol	11.48	0.24
12	citronellyl 2-methylpropanoate	11.79	7.53
13	trans-sabinene hydrate acetate	12.21	0.47
14	geranyl acetate	12.81	22.50
15	γ-cadinene	12.99	0.89
16	β-caryphyllene	13.54	1.72
17	α-humulene	13.96	0.88
18	β-caryphyllene oxide	15.40	0.26
19	1-heptadecene	16.54	3.76
20	citronellyl propionate	27.21	0.31

[a]Numbering refers to the elution order on a HP-5MS column.
[b]Retention index on a HP-5MS column.
[c]Peak area (%) was related to total detected compounds by GC-MS.

were previously reported for their antimicrobial activity against *S. mutans* with the MIC values of 0.8 mg/ml [6], 1.6 mg/ml [23,24], 0.8 mg/ml [23], 1.6 mg/ml [6,7], 0.4 mg/ml [23], 0.4 mg/ml [23,24], 0.4 mg/ml [23], 1.6 mg/ml [7,23], 0.8 mg/ml [7,23], and 0.8 mg/ml [7,23], respectively. These results demonstrate that although *Z. piperitum* seeds contain various antimicrobial components, which could be isolated either by methylene chloride extraction or water distillation, the effective method to gain the antimicrobial ingredients from the seed is to prepare essential oils by water distillation.

In summary, we have investigated for the first time the antimicrobial activity of the essential oils prepared from seeds of *Z. piperitum* against an oral pathogen *S. mutans*. These results showed that the seed essential oil of *Z. piperitum* has a potential, as a natural product, for controlling dental caries.

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Table 5. The chemical composition of the essential oil (CS-SD) from *Zanthoxylum piperitum* seed

Peak no.[a]	Compound	RI[b]	Peak area (%)[c]
1	sabinene	4.95	1.57
2	2-β-pinene	5.57	0.34
3	1-β-pinene	5.76	8.40
4	γ-phellandrene	5.98	0.90
5	γ-terpinene	6.42	21.41
6	trans-β-ocimene	6.61	3.14
7	terpinolene	7.37	2.13
8	linalool	7.42	1.55
9	cis-p-2-methanol-1-ol	7.84	0.90
10	citronellal	8.36	13.67
11	isopulegol	8.51	1.01
12	12-cyclohexene-1-one	8.74	3.03
13	terpinene-4-ol	8.81	0.45
14	β-fenchyl alcohol	8.98	1.52
15	cis-piperitol	9.11	0.45
16	trans-carvenol	9.40	0.22
17	citronellol	9.62	3.69
18	cuminic aldehyde	9.66	0.67
19	piperitone	9.92	1.01
20	geraniol	10.02	0.90
21	linalyl acetate	10.11	1.35
22	phellandral	10.33	2.24
23	benzene methanol	10.52	0.45
24	11,3-isobenzofurandione	11.28	0.89
25	citronellyl acetate	11.71	5.60
26	neryl acetate	11.82	0.67
27	methyl cinnamate	11.98	0.56
28	lavandulyl acetate	12.23	12.44
29	α-copaene	12.43	0.22
30	cyclotetradecane	12.60	0.01
31	3-ethoxyl-4-methyl exyphenol	12.76	0.22
32	β-caryophyllene	13.12	1.35
33	α-humulene	13.62	0.78
34	γ-cardinene	13.72	0.01
35	β-cubebene	14.00	0.45
36	caryophyllene oxide	15.41	0.01
37	hexadecane	15.78	0.01
38	δ-cadiene	16.20	0.67
39	T-muurolol	16.37	0.69
40	8-heptadecene	16.86	1.03
41	farnesyl acetate	18.73	0.01
42	naphthalene	20.08	0.01
43	14-(3,7,-dimethyl-6-o-octenyl)	20.97	1.58
44	(+,-)-(2)-dihydrofarnesal	21.12	0.44

[a]Numbering refers to the elution order on a HP-5MS column.
[b]Retention index on a HP-5MS column.
[c]Peak area (%) was related to total detected compounds by GC-MS.

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초록 : *Zanthoxylum piperitum* 종자의 치아우식균 *Streptococcus mutans*에 대한 항균활성

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치아우식균으로 알려져 있는 *Streptococcus mutans*에 대한 초피(*Zanthoxylum piperitum*)의 항균활성을 조사하기 위해, 뿌리, 줄기, 잎, 과피, 그리고 종자 부분을 methanol, ethyl acetate, hexane, methylene chloride등의 유기용매로 추출한 후 그 추출물의 항균활성을 1~3 mg/disc 농도범위에서 측정하였다. 그 결과, 종자의 경우만 활성이 나타났으며 특히 methylene chloride 추출물에서 비교적 높은 항균활성이 확인되었다. 초피종자의 항균활성이 종자의 정유성분을 추출할 경우에도 회수되는지를 조사하기 위해 초피종자 및 초피과피로부터 정유성분을 추출하고 이들의 *S. mutans*에 대한 항균활성을 측정하였다. 이때 두 정유성분 모두는 종자의 methylene chloride 추출물보다 더 강한 항균효과를 보였다. 이 정유성분들의 *S. mutans*에 대한 최소저해농도(MIC)를 액체배양으로 측정한 결과, 초피종자 정유성분의 MIC는 0.3 mg/ml 로, 또한 초피과피 정유성분의 MIC는 4 mg/ml로 나타났다. *S. mutans*에 대한 항균활성이 가장 높게 나타난 종자의 정유성분을 박층크로마토그래피 방법으로 7가지 분획으로 나누어 그 항균효과를 서로 비교 조사한 결과, 다섯번째 분획(CS-SD-E)의 항균활성이 다른 분획들의 항균활성에 비해 상대적으로 높았으나, 분획 전의 정유성분에 비해서는 모두 항균활성이 낮았다. 초피종자의 항균활성성분을 조사하기 위해, 초피종자의 methylene chloride 추출물과 초피종자 정유성분을 GC-MS로 비교 분석하였다. 초피종자의 methylene chloride 추출물에서는 *S. mutans*에 대한 항균활성이 이미 보고된 물질로서 carvacrol (0.24%), β-caryphyllene (1.72%), α-humulene (0.88%)등이 검출되었고, 초피종자 정유성분에서는 sabinene (1.57%), linalool (1.55%), citronellal (13.67%), terpinene-4-ol (0.45%), citronellol (3.69%), geraniol (0.9%), linalyl acetate (1.35%), β-caryophyllene (1.35%), α-humulene (0.78%) 및 δ-cadinene (0.67%) 등이 *S. mutans*에 대한 항균활성이 보고된 바 있는 물질로서 확인되었다. 이상의 연구결과는 초피종자가 *S. mutans*에 대해 항균활성을 가지며, 수증기 증류를 통해 확보하는 종자의 정유성분 회수가 이러한 항균물질을 추출하는 효과적인 방법임을 시사한다. 아울러 본 연구결과는 초피종자 유래의 정유성분이 *S. mutans*에 의해 발생하는 치아우식의 예방에 활용될 수 있음을 시사한다.