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Research Article

Antibacterial Activity of *Thuja occidentalis*, *Thuja orientalis* and *Chamaecyparis obtusa*

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ABSTRACT
The antibacterial activity of three Cupressaceae plants (*Thuja occidentalis*, *Thuja orientalis* and *Chamaecyparis obtusa*) was tested against three bacteria using the agar diffusion method. The ether and ethylacetate fraction of crude methanol extract from the three plants showed potent antibacterial activity against the tested microorganisms. The result showed that *Staphylococcus aureus* revealed the most sensitivity among the tested bacteria. *Thuja occidentalis* ether fraction and *Thuja orientalis* hexane fraction exhibited the highest antibacterial activity against *Staphylococcus aureus*. *E. coli* was shown the highest MIC values compared to the other two tested bacteria, which indicates the lowest antibacterial activity against the bacterium. This study promises an interesting future for designing a potentially active antibacterial agent from the three Cupressaceae plants.

Keywords: Antibacterial activity, *Thuja occidentalis*, *Thuja orientalis*, *Chamaecyparis obtuse.*

INTRODUCTION

Scientist have focused on the antimicrobial ability of plant-origin extracts since the 1990s for prolongation of shelf life and enhancement of food quality and decrease of pathogen without introduction of alien chemicals[1]. Plants have been used medicinally all over the world for many countries[2]. Approximately 60~80% of the world's population still relies on traditional medicines for the treatment of common illnesses[3]. Despite of tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to the public health. *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas fluorescens* which causes frequent infections, are the most common organisms used as an indicator of antibacterial[4].

Thuja occidentalis L. has been used for the treatment of bronchial catarrh, rheumatism, psoriasis, amenorrhea, cystitis and as an abortifacient, antidiarrheal, and hepatoprotective drug in folk medicine[5]. The previous research has revealed that the plant extracts possess antibacterial, antiviral, anti-inflammatory, anticancer, antispasmodic, gastroprotective[6]. *Thuja orientalis* (L.) Endl. has been used in traditional medicines for the treatment of haemorrhages, dermatitis, gout and chronic tracheitis and has been known that it has an anti-inflamatory[7,8]. *Chamaecyparis obtusa* (Sieb.et zucc.) Endl., commonly known as hinoki in Japan, has revealed that it contains terpenoids, lignan, flavonoid, and some of them are considered to possess antitumor, antimalarial and antibacterial activities[9-12]. In the current study, we evaluated the antibacterial activity of the fractions obtained from the *Thuja occidentalis*, *Thuja orientalis* and *Chamaecyparis obtusa*.

MATERIALS AND METHODS

Plant materials

The fresh leaves of three plants were collected in June 2010 from the Sunchon National University campus and authenticated by a taxonomist at Sunchon National University, Suncheon, Korea, where a voucher sample had been deposited. The collected plant leaves were air-dried in shadow for two weeks.

Bacterial strains and conditions

The hexane, ether, ethylacetate and water fractions of the three plants against the following bacteria: *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas fluorescens*. The bacteria were cultured on nutrient broth agar.

Plant extracts for antibacterial activity

100 gram of each plant sample was soaked in 1000ml methanol at room temperature for 24hrs. The solution was filtered through Whatman No.2 filter paper. The crude methanol extract was partitioned with 500ml of hexane and then the layer was concentrated (hexane fraction). The remaining layer was successively fractionated with 500ml ether and then ethylacetate (ether and ethylacetate fraction). The remaining residue was the water fraction. Each fraction was concentrated *in vacuo* to 30ml at 30°C and tested for antibacterial activity. Antibacterial activity was measured with the four fractions.

Determination of antibacterial activity

Each bacterial strain was grown in a nutrient broth at 30°C for 24h prior to testing. For the agar diffusion method, 0.1ml of the bacterial cell suspensions were poured uniformly on the nutrient broth agar plate. The paper disks

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Table 1: Antimicrobial activities against *Staphylococcus aureus* of each fractions of methanol extract from *Thuja occidentalis*, *Thuja orientalis* and *Chamaecyparis obtuse.*

Tested plant & fraction	Clear zone at various concentrations (mg/ml)							MIC (mg/ml)
	0.1	0.25	0.5	1.0	1.5	2.0	3.0	
Thuja occidentalis								
Hexane	10.5±1.3b	13.3±0.6a	13.7±0.9a	13.7±1.3a	12.7±0.4a	13.2±0.4a	13.6±0.9a	0.1
Ether	nd	10.0±1.8c	10.2±1.7c	14.2±1.1b	14.5±0.7b	16.8±1.0a	17.1±2.7a	0.25
Ethylacetate	8.9±0.1c	10.0±0.4c	12.6±0.9b	13.8±1.7a	14.9±1.3a	15.3±1.1a	15.1±1.2a	0.1
Water	nd	nd	nd	nd	nd	nd	nd	nd
Thuja orientalis								
Hexane	12.9±0.4b	18.8±0.5a	18.4±0.3a	17.7±0.6a	17.8±0.3a	17.6±0.7a	17.1±0.8a	0.1
Ether	8.6±0.1b	10.5±2.2b	13.1±1.5a	11.0±0.8b	11.9±0.3b	11.0±0.9b	11.1±0.4b	0.1
Ethylacetate	nd	8.7±0.6b	6.4±5.5b	10.2±1.4ab	11.3±1.2a	11.7±1.2a	12.0±1.2a	0.25
Water	nd	nd	nd	nd	nd	nd	nd	nd
Chamaecyparis obtusa								
Hexane	nd	10.6±1.5b	12.2±1.1a	11.2±0.3a	11.0±1.6a	11.0±0.2a	11.6±0.2a	0.25
Ether	nd	9.1±0.4b	12.5±1.2b	12.1±0.6b	12.4±2.1b	15.4±0.9a	16.0±1.1a	0.25
Ethylacetate	8.9±0.4b	10.6±0.3b	12.1±0.1a	11.6±2.6a	12.1±2.9a	13.1±3.2a	13.6±3.5a	0.1
Water	nd	nd	nd	nd	nd	nd	nd	nd

Data are mean ± SD of three individual determinations. Means with different letters in the same row indicate significant ($p < 0.05$) difference among concentrations tested.
nd: not detected.

Table 2: Antimicrobial activities against *Escherichia coli* of each fractions of methanol extract from *Thuja occidentalis*, *Thuja orientalis* and *Chamaecyparis obtuse.*

Tested plant & fraction	Clear zone (mean ± S.D., mm) at various concentrations (mg/ml)							MIC (mg/ml)
	0.1	0.25	0.5	1.0	1.5	2.0	3.0	
Thuja occidentalis								
Hexane	nd	nd	nd	nd	nd	nd	nd	nd
Ether	nd	nd	nd	nd	8.8±0.3a	9.9±0.7a	9.6±0.6a	1.5
Ethylacetate	nd	nd	nd	8.9±0.2b	9.3±0.2a	9.6±0.2a	10.1±.5a	1.0
Water	nd	nd	nd	nd	nd	9.1±0.3a	9.3±0.1a	2.0
Thuja orientalis								
Hexane	nd	nd	nd	nd	nd	nd	nd	nd
Ether	nd	nd	nd	8.8±0.1a	8.2±0.2a	8.8±0.1a	8.8±0.2a	1.0
Ethylacetate	nd	nd	nd	8.9±0.2a	9.2±0.4a	9.6±0.4a	9.9±0.3a	1.0
Water	nd	nd	nd	nd	8.7±0.1a	8.8±0.1a	9.2±0.3a	1.5
Chamaecyparis obtusa								
Hexane	nd	nd	nd	nd	nd	9.1±0.7a	9.3±0.2a	2.0
Ether	nd	nd	nd	nd	8.7±0.1a	9.1±0.2a	9.1±0.1a	1.5
Ethylacetate	nd	nd	nd	8.7±0.6a	8.9±0.4a	9.2±0.2a	9.5±0.5a	1.0
Water	nd	nd	nd	nd	nd	nd	nd	nd

Data are mean ± SD of three individual determinations. Means with different letters in the same row indicate significant ($p < 0.05$) difference among concentrations tested.
nd: not detected.

containing the fractions were carefully placed on the seeded Petri dishes. The diameters of inhibition zones were measured in millimeters after the strains were incubated at 28°C for 24h or 48h. And the minimum inhibitory concentration (MIC) was determined as the lowest concentration that caused an inhibition zone. Concentrations of the extract were 0.1, 0.25, 0.5, 1.0,1.5, 2.0 and 3.0 mg/ml.

Statistical analysis

The experiments were conducted in triplicate and the results are expressed as mean ± standard deviation (SD). Differences between means were tested through Duncan's multiple range test. Statistical analysis was performed with the software program SPSS (Version 22.0).

RESULTS AND DISCUSSION

Table 3: Antimicrobial activities against *Pseudomonas fluorescens* of each fractions of methanol extract from *Thuja occidentalis, Thuja orientalis* and *Chamaecyparis obtuse.*

Tested plant & fraction	Clear zone (mean ± S.D., mm) at various concentrations (mg/ml)							MIC (mg/ml)
	0.1	0.25	0.5	1.0	1.5	2.0	3.0	
Thuja occidentalis								
Hexane	nd	nd	nd	nd	nd	nd	nd	nd
Ether	nd	nd	8.7±0.1b	8.7±0.3b	9.0±0.8ab	9.3±0.1a	9.5±0.2a	0.5
Ethylacetate	nd	nd	nd	9.2±0.2a	9.6±0.2a	9.6±0.3a	10.2±0.8a	1.0
Water	nd	nd	nd	nd	nd	nd	nd	nd
Thuja orientalis								
Hexane	nd	nd	nd	nd	nd	nd	nd	nd
Ether	nd	nd	8.6±0.1a	8.8±0.1a	8.7±0.2a	9.4±0.2a	9.1±1.0a	0.5
Ethylacetate	nd	nd	nd	8.8±0.1b	9.1±0.2ab	9.8±0.6a	10.7±0.3a	1.0
Water	nd	nd	nd	nd	nd	9.2±0.4a	8.8±0.2b	2.0
Chamaecyparis obtusa								
Hexane	nd	nd	nd	nd	9.1±0.1a	9.1±0.3a	9.1±0.3a	1.5
Ether	nd	nd	8.8±0.1a	8.9±0.3a	9.8±0.3a	9.7±0.7a	9.8±0.4a	0.5
Ethylacetate	nd	nd	nd	8.9±0.1b	9.4±0.1ab	9.7±0.3a	10.5±0.2a	1.0
Water	nd	nd	nd	nd	10.5±0.5a	9.8±1.0a	9.5±0.2a	1.5

Data are mean ± SD of three individual determinations. Means with different letters in the same row indicate significant ($p < 0.05$) difference among concentrations tested.
nd: not detected.

The inhibitory activity of different concentration of four fraction from the three Cupressaceae plants on growth of *Staphylococcus aureus* is represented in Table 1. The water fraction of the three plants did not excute the growth inhibition to *S. aureus* at any tested concentrations. Among the tested fraction, ether fraction of *T. occidrntalis* and *C. obtusa* and hexane fraction of *T. orientalis* was observed to perform higher antibacterial activity on *S. aureus*. The minimum inhibitory concentration for hexane, ether and ethylacetate fracrion of the three plants was 0.1 or 0.25mg/ml for *S. aureus*. The fractions from the three plants exhibited weak inhibitory activity for *Escherichia coli* (Table 2). Especially, none of the hexane fraction of *T. occidentalis* and *T. orientalis* and water fraction of *C. obtusa* showed any activity against *E. coli*. Among the investigated fractions, the ethylacetate fraction of the three plants exhibited the highest inhibitory activity on *E. coli*. Results obtained for the antimicrobial tests performed on the four fracrion of the three plants against *Pseudomonas flurescens* are presented in Table 3. None of the hexane fraction of *T. occidentalis* and *T. orientalis* showed activity against *P. fluorescens*. Generally, the ethylacetate fraction among the investigated fractions exhibited the highest antibacterial activity followed by the ether fraction. The lowest minimum inhibitory concentration was observed for ether fraction of the three plants against *P. fluorescens*. The two *Thuja* species fractions showed higher antibacterial activity against *S. aureus* and *E. coli* than *C. obtusa* fractions, but on the other hand *Pseudomonas flurescens* was more susceptible to the fractions of *C. obtusa* than those of the two *Thuja* species. The measurement of MIC indicated that *Staphylococcus aureus* is the sensitive microorganism with the lowest MIC value. Finding from the present study also showed that *Thuja orientalis* fractions gave the lowest MIC values.

The comparison of the antibacterial activity of the three plants of Cupressaceae described here for the first time. It showed promising activity against *Staphylococcus aureus* among the tested bacteria. *Staphylococcus aureus* (a Gram-positive bacterium) was found susceptible to various plant extracts evaluated in different studies such as methanol extract of *Datura innoxia*[13], some mecinal plants of the island Soqotra[14], *Verbascum* species[15], *Artemisia parviflora*[16] and the tested fractions was found to be the weakest activity against *E. coli*. This finding was different with that *Pilea symmeria* was the most effective against *E. coli* and *Staphylococcus aureus*[17].

CONCLUSION

The three Cupressaceae plants are potential sources of bioactive compounds that exhibit antibacterial activity. Of the special interest is the potency of ether and ethylacetate fraction of the methanol extract, which show strong antibacterial activity. The further isolation and identification of the individual constituents present in the fractions is needed.

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