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Antimicrobial activity and chemical components of two plants, *Artemisia capillaris* and *Artemisia iwayomogi*, used as Korean herbal Injin

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This study compared the antimicrobial activity and chemical composition of two plants used as "Injin" (Korean herbal medicine), namely, Injinho (*Artemisia capillaris* Thunberg) and Haninjin (*Artemisia iwayomogi* Kitamura). The ethyl acetate and ether fractions of crude methanol extracts from *A. capillaris* and *A. iwayomogi* were tested against three gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*), two gram-negative bacteria (*Escherichia coli*, *Pseudomonas fluorescens*), and a yeast (*Saccharomyceus cerevisiae*). The antimicrobial activity of the ethyl acetate and ether fraction of both plants was strong, but that of *A. iwayomogi* extracts was higher than that of *A. capillaris* extract for the microbes tested. The minimum inhibitory concentration of the ether and ethyl acetate fraction of *A. iwayomogi* was highest for *P. fluorescens* and lowest for *S. aureus* and *E. coli*. We analyzed the chemical composition of the ethyl acetate fraction of *A. capillaris* and *A. iwayomogi* using gas chromatography-mass spectrometry. The main components of *A. capillaris* and *A. iwayomogi* were escoparone (86.82%) and scopoletin (20.47%), respectively.

Key words: antimicrobial activity, *Artemisia capillaris*, *Artemisia iwayomogi*, escoparone, scopoletin

INTRODUCTION

Allelopathy is defined as any direct or indirect harmful effect of a plant on another organism (including microorganisms) through production of chemical (Rice 1984). Secondary metabolites may function in plant defense via allelopathic processes (Fernandez et al. 2009).

In recent years, there has been increasing interest in healthy lifestyles and healthy aging. As a result, many people are involved in searches for natural compounds that can improve health, especially those of plant origins. A great number of aromatic, spicy, medicinal, and other plants belonging to the family Asteraceae contain chemical compounds exhibiting antimicrobial and antioxidant properties (Boussaada et al. 2008). Antimicrobial and antioxidative plant oils and extracts have been used for many purposes, including raw and processed food preservation, pharmaceuticals, alternative medicines, and natural therapies (Hammer et al. 1999). Natural products are perceived as having fewer negative impacts than synthetic agents; natural products may be effective, selective, biodegradable, and less toxic to the environment. The genus *Artemisia* is one of the most important genera in the family Asteraceae and is widespread throughout the world. About 30 species in this genus are found in Korea. Members of this genus have a characteristic scent or taste, and are of botanical and pharmaceutical

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interest (Kordali et al. 2006) because they contain active polyphenolic compounds (Schmidt et al. 2007). Aromatic *Artemisia* plants are used as spices and in folk remedies as antiseptics. For example, powdered leaves of *A. absinthium*, *A. biennis*, *A. frigida*, and *A. ludoviciana* have been applied externally in salves and washes by native North Americans to treat sores and wounds and internally to treat chest infections (Kershaw 2000). Though *A. capillaris* and *A. iwayomogi* are considered weeds, the plants have been used in traditional medicine in Korea as Injin. *A. capillaris* is a common perennial herb and has been cultivated for use as a treatment for hepatitis in Korea. *A. capillaris* has antifungal effects (Choi et al. 2005) and allelopathic effects (Kil 1999), stimulates immune activities in human cells, and has anticancer activity (Lee et al. 2004). The major active components of *A. capillaris* are scoparone and capillarisin, and the concentrations of these compounds are related to the season of harvest (Choi et al. 2007). *A. iwayomogi* is a unique shrub of the genus in Korea. It inhibits hepatotoxicity and liver cirrhosis (Song et al. 2001) and has antioxidant activity (Kim et al. 1997) as well as antitumor and immunomodulating activity (Koo et al. 1994). The aim of this study was to compare the antimicrobial activity and major components of *A. capillaris* and *A. iwayomogi*.

MATERIALS AND METHODS

Plant material

The aerial parts of *A. capillaris* and *A. iwayomogi* were collected from a cultivated population in the Jinan Medicinal Herbs Experiment Station, Jeollabuk-do Agricultural Research & Extension Services (35°46′15″ N, 127°22′40″ E), Korea in June 2007. The leaves were air-dried for 12-14 days after collection for antimicrobical activity tests and chemical analysis.

Microorganisms

The test microorganisms included three gram-positive bacteria (*Bacillus cereus* ATCC 27348, *Bacillus subtilis* ATCC 9327 and *Staphylococcus aureus* ATCC 13301), two gram-negative bacteria (*Escherichia coli* ATCC 15489 and *Pseudomonas fluorescens* ATCC 11250), and one yeast (*Saccharomyces cerevisiae* IFO 1950). The gram-positive and gram-negative bacteria were cultured on a nutrient broth agar, while the yeast was cultured on yeast malt broth agar.

Extract preparation for antimicrobial activity of two *Artemisia* plants

We soaked 200 g samples of air-dried leaves of *A. capillaris* and *A. iwayomogi* in 1,000 mL of methanol and ground the mixture for 20 minutes. The solution was kept at room temperature for 30 minutes and then filtered through Whatman No. 2 paper.

The crude methanol extract was partitioned with 500 mL of hexane and then the top layer was concentrated (comprising the hexane fraction). The remaining layer was successively fractionated with 500 mL of diethyl ether and then ethyl acetate (forming the ether and ethyl acetate fractions). The remaining residue was the water fraction. Each fraction was concentrated *in vacuo* to 30 mL at 30°C and tested for antimicrobial activity. Antimicrobial activity was measured only with the ether fraction and the ethyl acetate fraction.

Determination of antimicrobial activity

Each bacterial strain was grown in a nutrient broth at 30°C for 18-24 hours prior to testing and subcultured three times for another 18-24 hours. The turbidity of bacterial cell suspensions was brought to 0.3 optimal density (OD) at 660 nm by adding sterile broth and was then used for the tests. We poured 0.1 mL of the bacterial cell suspensions uniformly on nutrient broth agar plates. The paper disks containing the extracts (ether fraction and ethyl acetate fraction) were carefully placed on the seeded Petri dishes. The diameters of the resulting inhibition zones were measured in mm after the cultures were incubated at 30°C for 24 hours or 48 hours (Bauer et al. 1966). The antimicrobial activity was calculated as the net zone of inhibition estimated from the growth inhibition zone measurements (Mahasneh and El-Oqlah 1999). The minimum inhibitory concentration (MIC) was determined as the lowest concentration that caused an inhibition zone. We measured the inhibition caused by 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0 mg/mL extracts.

Gas chromatography-mass spectrometry (GC-MS)

The ethyl acetate fractions of *A. capillaris* and *A. iwayomogi* leaf extracts were analyzed by GC-MS using a GC–MSD, equipped with an Ultra 2 (Crosslinked 5% PH ME Siloxane, 25 m length × 0.20 mm i.d. Hewlett Packard, Palo Alto, CA, USA) with helium as a carrier gas at a constant flow rate of 1.0 mL/min. One μL of the extract was

injected into the column using the split injection mode with a 10:1 split ratio. The oven temperature was initially held at 100°C, then raised to 280°C at a rate of 3°C/min for 50 minutes, and finally held at 280°C for 5 minutes. The temperatures of the injector and detector were 200°C and 240°C, respectively. The mass detector was operated in the electron impact mode with an ionization energy of 70 eV, a scanning range of 33-550 a.m.u., and a scan rate of 1.4 scans/s. Components of the extracts were identified using the Wiley 275 Imass spectral database (Hewlett-Packard, 1995) or by manual interpretation. Glutaric acid was used as an internal standard for quantification.

Data analysis

We used a randomized complete block design with four replications in all experiments. Each experiment was repeated three or four times. Statistical analysis was conducted using the software program SPSS ver. 10.0 (SPSS Inc., Chicago, IL, USA). Comparisons between treatments were made at the 0.05 level using Duncan's multiple-range test.

RESULTS AND DISCUSSION

Antimicrobial activity of the two *Artemisia* plants

Local people use different plant parts to prepare phytomedicines, and the most frequently used parts are aerial parts, including leaves, fruits, seeds, and flowers (Kültür 2007). The antimicrobial activity and MIC of the ether and ethyl acetate fractions of methanol extract from the two *Artemisia* plants are shown in Tables 1 and 2. In general, *A. iwayomogi* exhibited stronger antimicrobial activity than *A. capillaris*, and the ethyl acetate

Table 1. Antimicrobial activity of the ether fraction of methanol extracts from *Artemisia capillaris* and *Artemisia iwayomogi*[*]

	Clear zone (± SD, mm) at various concentrations (mg/mL)							MIC (mg/mL)
	0.10	0.25	0.50	1.00	1.50	2.00	3.00	
Bacillus cereus								
A. capillaris	–[†]	–	–	–	8.3 ± 0.1^{b}	8.7 ± 0.2^{ab}	9.5 ± 0.1^{a}	1.5
A. iwayomogi	–	–	8.8 ± 0.1^{d}	9.6 ± 0.1^{d}	11.1 ± 0.1^{c}	12.1 ± 0.1^{b}	14.1 ± 0.1^{a}	0.5
Bacillus subtilis								
A. capillaris	–	–	8.3 ± 0.1^{c}	8.9 ± 0.4^{bc}	9.4 ± 0.2^{b}	9.7 ± 0.1^{b}	11.1 ± 0.1^{a}	0.5
A. iwayomogi	–	–	–	9.3 ± 0.5^{c}	12.1 ± 0.4^{b}	12.8 ± 0.6^{b}	14.0 ± 0.4^{a}	1.0
Staphylococcus aureus								
A. capillaris	–	–	–	–	–	8.1 ± 0.1^{a}	9.4 ± 0.2^{a}	2.0
A. iwayomogi	–	–	–	8.5 ± 0.1^{c}	10.3 ± 0.2^{b}	11.3 ± 0.3^{a}	11.9 ± 0.1^{a}	1.0
Escherichia coli								
A. capillaris	–	–	–	–	8.4 ± 0.2^{b}	9.1 ± 0.1^{b}	10.2 ± 0.2^{a}	1.5
A. iwayomogi	–	–	–	8.6 ± 0.1^{c}	9.4 ± 0.2^{c}	10.8 ± 0.8^{b}	12.4 ± 0.6^{a}	1.0
Pseudomonas fluorescens								
A. capillaris	–	–	–	–	–	–	8.6 ± 0.2^{a}	3.0
A. iwayomogi	–	–	–	–	–	–	8.6 ± 0.1^{a}	3.0
Saccharomyces cerevisiae								
A. capillaris	–	–	–	8.7 ± 0.1^{c}	9.3 ± 0.1^{bc}	9.8 ± 0.1^{b}	11.1 ± 0.1^{a}	1.0
A. iwayomogi	–	–	–	–	8.8 ± 0.0^{a}	9.1 ± 0.1^{a}	9.3 ± 0.1^{a}	1.5

SD, standard deviation; MIC, minimum inhibitory concentration.
[*]Means with the same letters within a row are not significantly different at $P = 0.05$, according to Duncan's multiple range test.
[†]Means are not detected.

fraction showed stronger antimicrobial activity than the ether fraction (Tables 1 and 2). The diameters of the clear zones resulting from application of the ethyl acetate fraction ranged from 8.8 mm to 19.9 mm (including the diameter of the disk, 8.0 mm). We classified the antimicrobial activity of the plant extracts into three classes as follows: weak (< 10 mm inhibition zone), moderate (10-15 mm inhibition zone), and good to very good (> 15 mm inhibition zone) (Mahasneh 2002).

Some gram-negative bacteria are less sensitive than other microbes to the action of plant extracts and compounds (Boussaada et al. 2008, Yun et al. 2008), but gram-negative bacteria are often more susceptible than gram-positive bacteria to the inhibitory effects of essential oils (Smith-Palmer et al. 1998). In our study, the ether and ethyl acetate fractions of the plant extracts were more active against gram-positive bacteria than gram-negative bacteria and yeast: the antimicrobial action of the ethyl acetate fraction of *A. iwayomogi* was good to very good against all three gram-positive bacteria. Similar results have been reported other species in the family Asteraceae (e.g., Boussaada et al. 2008, Yun et al. 2008). *B. subtilis* was the microorganism with the lowest MIC value, indicating that it was the most sensitive to the antimicrobial properties of the plant extracts. Other sensitive microorganisms included *B. cereus* and *E. coli*, *S. cerevisiae* (yeast) exhibited higher sensitivity to the *A. capillaris* fractions than the *A. iwayomogi* fractions. Yeast is also resistant to the action of several other plant extracts (Seo et al. 2008).

The chemical composition

The ethyl acetate fraction of extracts of *A. capillaris* and *A. iwayomogi* leaves with a yield of 0.39% and 1.00%, respectively. The chemical compositions of the ethyl acetate fractions of *A. capillaris* and *A. iwayomogi* are sum-

Table 2. Antimicrobial activity of the ethyl acetate fraction of methanol extract from *Artemisia capillaris* and *Artemisia iwayomogi*[*]

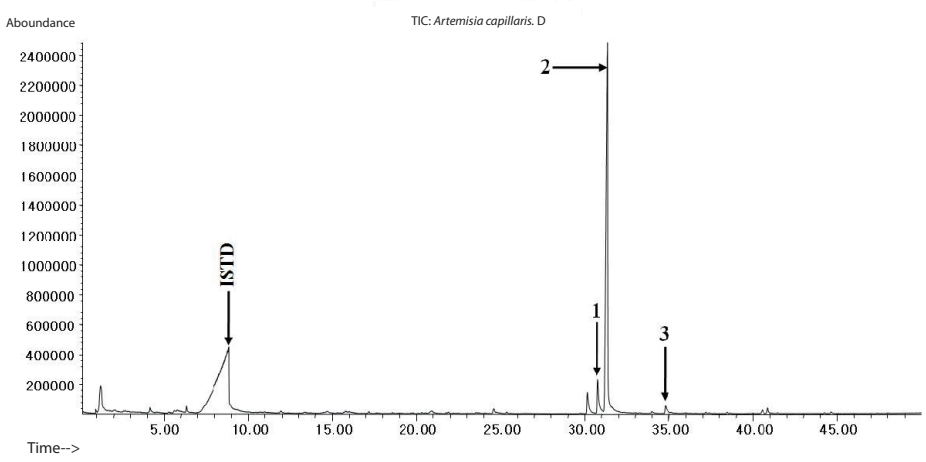
	Clear zone (± SD, mm) at various concentrations (mg/mL)							MIC (mg/mL)
	0.10	0.25	0.50	1.00	1.50	2.00	3.00	
Bacillus cereus								
A. capillaris	–[†]	–	–	9.4 ± 0.6^{b}	9.7 ± 0.1^{ab}	10.2 ± 0.3^{a}	10.7 ± 0.3^{a}	1.0
A. iwayomogi	–	9.8 ± 0.4^{e}	12.8 ± 0.4^{d}	16.2 ± 0.4^{c}	17.1 ± 0.4^{bc}	17.7 ± 0.3^{bc}	18.3 ± 0.6^{a}	0.25
Bacillus subtilis								
A. capillaris	–	–	9.0 ± 0.1^{c}	9.7 ± 0.7^{bc}	10.0 ± 1.7^{ab}	10.6 ± 1.6^{ab}	11.5 ± 1.7^{a}	0.5
A. iwayomogi	–	9.0 ± 1.1^{e}	13.6 ± 0.2^{d}	16.5 ± 1.9^{c}	17.3 ± 1.5^{c}	18.3 ± 1.1^{b}	19.9 ± 0.6^{a}	0.25
Staphylococcus aureus								
A. capillaris	–	–	–	8.3 ± 0.1^{a}	8.6 ± 0.1^{a}	9.1 ± 0.1^{a}	9.6 ± 0.1^{a}	1.0
A. iwayomogi	–	8.6 ± 0.1^{e}	11.2 ± 0.4^{d}	13.2 ± 0.5^{c}	14.2 ± 0.4^{b}	15.2 ± 1.0^{a}	16.1 ± 0.2^{a}	0.25
Escherichia coli								
A. capillaris	–	–	–	–	8.4 ± 0.1^{b}	9.2 ± 0.2^{ab}	9.6 ± 0.1^{a}	1.5
A. iwayomogi	–	8.8 ± 0.9^{f}	11.1 ± 2.4^{e}	12.9 ± 3.1^{d}	14.4 ± 3.0^{c}	16.1 ± 2.9^{b}	17.9 ± 2.5^{a}	0.25
Pseudomonas fluorescens								
A. capillaris	–	–	–	–	9.1 ± 0.1^{b}	9.4 ± 0.2^{b}	10.5 ± 0.1^{a}	1.5
A. iwayomogi	–	–	–	–	-	8.8 ± 0.1^{a}	9.7 ± 0.3^{a}	2.0
Saccharomyces cerevisiae								
A. capillaris	–	–	–	8.6 ± 0.1^{b}	9.0 ± 0.3^{b}	9.4 ± 0.4^{b}	10.5 ± 1.1^{a}	1.0
A. iwayomogi	–	–	–	9.0 ± 0.4^{b}	9.8 ± 0.4^{b}	10.5 ± 1.0^{ab}	10.9 ± 1.1^{a}	1.0

SD, standard deviation; MIC, minimum inhibitory concentration.
[*]Means with the same letters within a row are not significantly different at $P = 0.05$, according to Duncan's multiple range test.
[†]Means are not detected.

marized in Figs. 1 and 2. The main component of the *A. capillaris* extract was escoparone (86.82%). Escoparone may contribute to the anti-inflammatory activity of *A. capillaris* (Jang et al. 2005). The main component of *A. iwayomogi* was scopoletin (20.47%). Escoparone, scopoletin, and isofraxidin, which were identified in extracts of both *Artemisia* plants are phenolic compounds, while achillin, identified in the extract of *A. iwayomogi,* is a sesquiterpene lactone. Escoparone and scopoletin, the major phenolic constituents of the two *Artemisia* plants, may contribute to their antimicrobial activity. The differences identified in the chemical components of the two *Artemisia* plants are likely responsible for at least some of the differences in their antimicrobial activity. Our results should encourage more in-depth studies of the antimicrobial activity of the individual chemicals identified in the two *Artemisia* plants.

Most phenolics that display antimicrobial activity are phenolic acids or flavonoids. Phenolic acids are a major class of phenolic compounds occurring in a diverse range of plants (Wojdyło et al. 2007), and the phenolic moiety plays an important role in determining a plant's antimicrobial activity (Kujumgiev et al. 1993). And mixtures of phenolic acids and other organic compounds can cause inhibitory effects even when the concentrations of the individual compounds are well below inhibitory levels (Blum 1996).

Our results provide the first detailed documentation of the *in vitro* antimicrobial features of two *Artemisia* species used as Korean herbal Injin. The antimicrobial compounds in *Artemisia* plants may have potential for use as safe and eco-friendly bactericides.



No.	Chemical name	Peak area/ISTD area	% Area	MW	MF
1	Scopoletin	0.08	3.24	192.04	C10H8O4
2	Escoparone	0.88	86.82	206.06	C11H10O4
3	Isofraxidin	0.02	0.85	222.05	C11H10O5

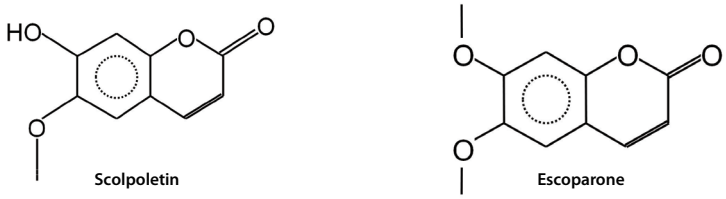
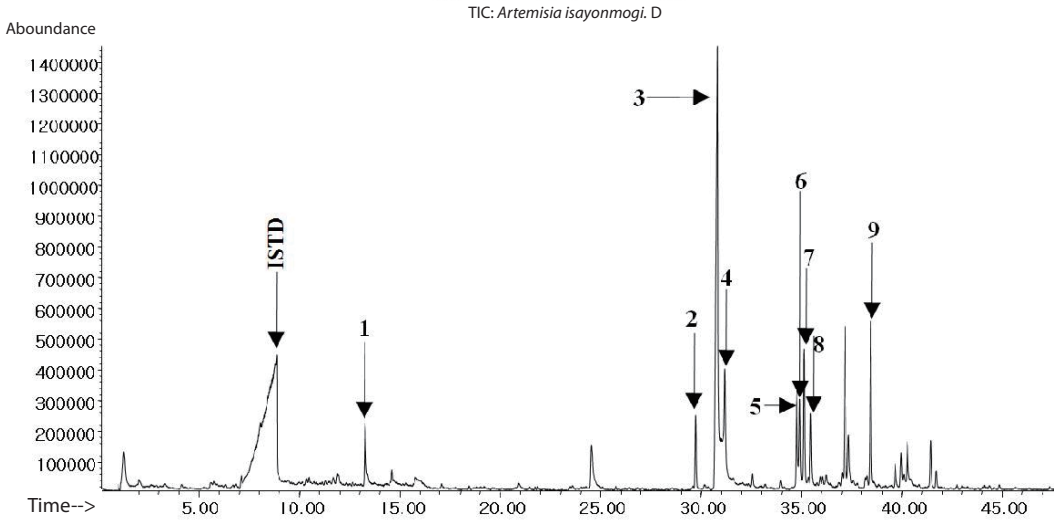


Fig. 1. GC chromatogram of the ethyl acetate fraction of an extract of *Artemisia capillaris* leaves and its components. GC, gas chromatography; ISTD, internal standard; MW, molecular weight; MF, molecular formula.



No.	Chemical name	Peak area/ISTD area	% Area	MW	MF
1	Coumarin	0.08	2.13	146.04	C9H6O2
2	Palmitic acid	0.07	1.87	270.26	C17H34O2
3	Scopoletin	0.79	20.47	192.04	C10H8O4
4	Escoparone	0.20	5.06	206.06	C11H10O4
5	Isofraxidin	0.13	3.44	222.05	C11H10O5
6	Linoleic acid	0.10	2.47	294.26	C19H34O2
7	Linolenic acid	0.14	3.66	294.24	C19H32O2
8	Neophytadiene	0.08	2.02	278.30	C20H38
9	Achillin	0.19	4.83	246.13	C15H18O3

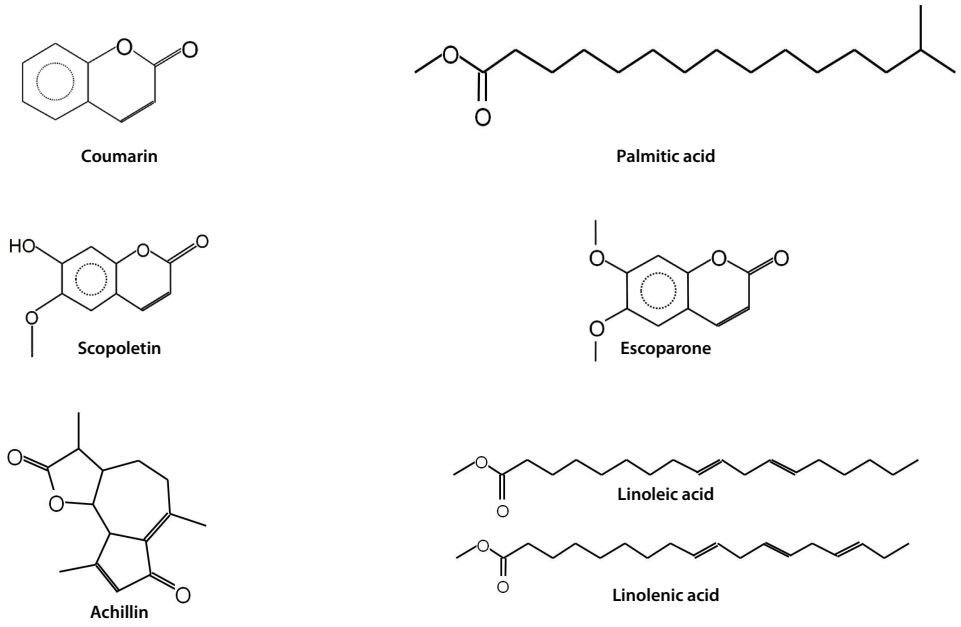


Fig. 2. GC chromatogram of ethyl acetate fraction of an extract of *Artemisia iwayomogi* leaves and its components. GC, gas chromatography; ISTD, internal standard; MW, molecular weight; MF, molecular formula.

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