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Antimicrobial Effect of the Wood Vinegar from *Cryptomeria japonica* Sapwood on Plant Pathogenic Microorganisms

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Abstract The antimicrobial effect of the wood vinegar of *C. japonica* sapwood and its constituents was evaluated against *Ralstonia solanacearum*, *Phytophthora capsici*, *Fusarium oxysporum*, and *Pythium splendens*. Phenols and guaiacols had a strong antimicrobial effect against four kinds of microorganisms, but methanol and acetic acid exhibited little or no antimicrobial activity.

Key words: *Cryptomeria japonica*, wood vinegar, antimicrobial activity, phenols, guaiacols

For prevention of contamination and damage caused by the overuse of agricultural chemicals and chemical fertilizers, organic-farming and chemical-free vegetable cultivations have been on the increase. As one of such environment-friendly agricultural methods, charcoal and wood vinegar prepared from wood are drawing people's attention.

The wood vinegar has been investigated to be utilized as not only a raw material of medicine, a food preservative, and an antioxidant, but also, in agricultural and environmental areas, an agent supplementary to agricultural chemicals, a soil conditioner, a compost fermentation accelerating agent, a weed control agent, and an agent accelerating the growth of plants and the development of roots [1–5]. The wood vinegars have been studied as a repellent and insecticide against insects injurious to crops [6–8] and as a antimicrobial agent for fungi, dermaptophyte, and bacteria [9–14].

The constituents of wood vinegars were reported from the xylem of black oak, mulberry, oak, sugi, bamboo, and the cortex of hardwoods, and varied dependent on both the kind of raw materials and the carbonization conditions [15–20]. Despite investigations, the use of wood vinegar is insufficient for agricultural use. Thus we assessed the antibacterial and antifungal effects of *C. japonica* (sugi) wood vinegar and its constituents against plant pathogenic microorganisms with the intention of evaluating agricultural supplement to antimicrobial chemicals.

Around 35-year-old *C. japonica* sapwood, belonging to the family Taxodiaceae, was collected from Tano forest science station of University of Miyazaki in Japan.

The wood sample ($10\times10\times100$ mm^3) was carbonized with an automatic tubular furnace ISUZU EKR-122K (Japan) under nitrogen gas at a rate of 20 ml/min. The temperature was elevated at a rate of 200°C/h up to 600°C and then kept at 600°C for 2 h. The evolution gas was cooled with a condenser at 4°C to give the brown liquid condensates.

After being cooled, the liquid was centrifuged at 2,500 rpm for 1 h and the supernatant was collected as wood vinegar.

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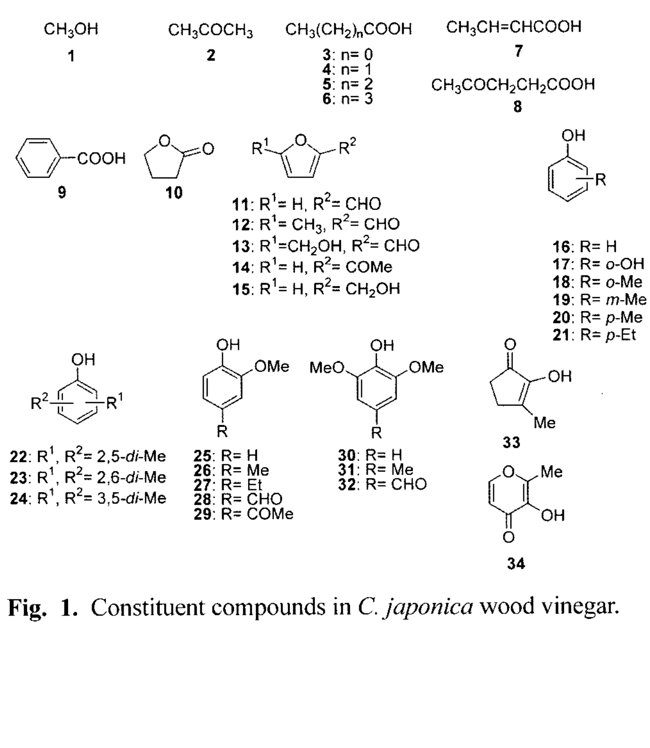

Fig. 1. Constituent compounds in *C. japonica* wood vinegar.

The wood vinegar obtained was at pH 2.0. Capillary GC chromatography of the wood vinegar was performed on Shimadzu GC-14B with FID detector under the same conditions as described previously [16].

Standard strains used were plant pathogenic microorganisms, *Ralstonia solanacearum* No. 8224, *Phytophthora capsici* CAF892, *Fusarium oxysporum* OK1, and *Pythium splendens* KU8601.

The wood vinegar and its constituent compounds, except for 3,5-xylenol and syringaldehyde, were dissolved in distilled water and were neutralized (pH 7.0) with 5 M sodium hydroxide; dimethyl sulfoxide was used for 3,5-xylenol and syringaldehyde as a solubilizing agent in water. The neutralized aqueous solutions were sterilized by filtration through a membrane filter (0.22 μm), and the resulting solutions were used as sample solutions. To make test medium, an appropriate amount of the sample solution was added to sterilized PSA and PDA. The PSA test medium was used for bacterium *R. solanacearum,* and the PDA test medium for other fungi. The microorganisms were implanted into the test media and cultured at 30°C for 7 days. Then, it was observed whether or not colonies were formed and the minimum inhibitory concentration (MIC) was determined.

The result of determination of wood vinegar constituents is shown in Fig. 1 and Table 1. The wood vinegar contained methanol 1 (15.34 mg/ml), acetone 2 (0.54 mg/ml), carboxylic acids 3–10 (43.77 mg/ml), furans 11–15 (2.34 mg/ml), phenols

Table 1. Inhibitory effects of *C. japonica* wood vinegar and its constituents against plant pathogenic microorganisms.

Constituent	Content mg/ml	MIC mg/ml			
		R. solanacearam	*P. capsici*	*F. oxysporum*	*P. splendens*
Neutralized wood vinegar (pH 7.0)	-	5.00	10.00	20.00	20.00
Methanol (**1**)	15.34	>5.00	>5.00	>5.00	>5.00
Acetone (**2**)	0.54	>5.00	>5.00	>5.00	>5.00
Acetic acid (**3**)	36.69	5.00	>5.00	>5.00	>5.00
Propionic acid (**4**)	2.25	5.00	>5.00	>5.00	>5.00
Butyric acid (**5**)	1.09[a]	>5.00	2.50	>5.00	5.00
Valeric acid (**6**)	0.06	>5.00	2.50	>5.00	5.00
Crotonic acid (**7**)	1.09	5.00	1.25	>5.00	1.25
Levulinic acid (**8**)	1.44	>5.00	>5.00	>5.00	>5.00
Benzonic acid (**9**)	0.06	5.00	0.16	5.00	0.63
γ-Butyrolactone (**10**)	1.09[a]	>5.00	>5.00	>5.00	>5.00
2-Furaldehyde (**11**)	1.78	1.25	>5.00	5.00	2.50
5-Methyl-2-furaldehyde (**12**)	0.31	>5.00	1.25	5.00	2.50
5-Hydroxymethyl-2-furaldehyde (**13**)	0.25	5.00	>5.00	>5.00	>5.00
2-Acetylfuran (**14**)	0	>5.00	2.50	5.00	5.00
Furfuryl alcohol (**15**)	0	>5.00	5.00	>5.00	5.00
Phenol (**16**)	1.50	1.25	1.25	1.25	0.31
Pyrocatechol (**17**)	1.88	0.31	1.25	2.50	0.31
o-Cresol (**18**)	0.33	0.31	1.25	1.25	0.63
m-Cresol (**19**)	0.33	0.31	0.31	0.63	0.63
p-Cresol (**20**)	0.72	0.31	0.31	0.31	0.63
p-Ethylphenol (**21**)	1.78[e]	1.25	0.31	0.63	0.16
2,5-Xylenol (**22**)	0.04	1.25	1.25	1.25	1.25
2,6-Xylenol (**23**)	0.02	2.50	2.50	1.25	1.25
3,5-Xylenol (**24**)	1.78[e]	0.31	0.31	0.16	0.31
Guaiacol (**25**)	3.76	1.25	2.50	2.50	1.25
4-Methylguaiacol (**26**)	2.60	1.25	1.25	1.25	1.25
4-Ethylguaiacol (**27**)	0.62	1.25	1.25	1.25	1.25
Vanillin (**28**)	0.46	0.63	2.50	5.00	5.00
4-Acetylguaiacol (**29**)	0.15	1.25	2.50	2.50	1.25
Syringol (**30**)	0	1.25	2.50	2.50	2.50
4-Methylsyringol (**31**)	0	1.25	1.25	1.25	1.25
Syringaldehyde (**32**)	0	1.25	2.50	2.50	2.50
Cyclotene (**33**)	2.66	5.00	5.00	5.00	5.00
Maltol (**34**)	0.38	0.63	2.50	5.00	1.25

[a] Butyric acid (**5**) and γ-butyrolactone (**10**) were not separated from each other under the present GC conditions and their contents were determined based on **10**.

16–24 (8.38 mg/ml), guaiacols 25–29 (7.59 mg/ml), cyclotene 33 (2.66 mg/ml), and maltol 34 (0.38 mg/ml) in their order. No syringols 30–32 were found in the *C. japonica* wood vinegar, although they were contained in the wood vinegar from hardwoods and oak [16].

Although the antimicrobial activities of wood vinegars from several plants have been examined against some microorganisms such as fungi, dermaptophyte, and bacteria [9–14], the MIC of the wood vinegars and their components against plant pathogenic microorganisms have not been reported. Therefore, we examined the MIC of each component in the *C. japonica* wood vinegar for the purpose of finding antimicrobial substances in the wood vinegar. The antimicrobial test was performed using 31 kinds of main constituents in the *C. japonica* wood vinegar, syringols, 4-methylsyringol, and syringaldehyde. The neutralized wood vinegars showed an MIC of 5.00 mg/ml against *R. solanacearum* and those of 10.00–20.00 mg/ml against three kinds of fungi.

In the wood vinegar used in this study, the contents of acetic acid and methanol were particularly high but their antimicrobial effect was extremely weak. Acetone and the other carboxylic acids had a weak antimicrobial effect. Matsuda *et al.* [21] reported that lower fatty acids such as acetic and propionic acids showed weak activities of growth inhibition against yeasts and bacteria (MIC 10.0–50.0 mg/ml at pH 7.0). Our results on the antimicrobial effects against the microorganisms almost coincided with theirs. The carboxylic acids in the neutralized *C. japonica* wood vinegar have weak antimicrobial activities and seem to have little inhibition effect on the microorganisms, because the total content of the carboxylic acids was about 0.88 mg/ml at the MIC (20 mg/ml) of the wood vinegar against the fungi. Benzoic acid showed an exceptional antimicrobial effect against two kinds of fungi, *P. capsici* and *P. splendens*. Benzoic acid is generally used as a food preservative. Because its content in the *C. japonica* wood vinegar is as low as 0.06 mg/ml, however, benzoic acid probably makes little contribution to the antimicrobial effect of the wood vinegar.

Phenols and guaiacols appeared to have strong antimicrobial effect in general, and showed an MIC of 1.25 mg/ml. Among the phenols, pyrocatechol, *o*-cresol, *m*-cresol, *p*-cresol, and 3,5-xylenol showed an MIC of 0.31 mg/ml, having strong antimicrobial activity against *R. solanacearum*. Among the guaiacols, vanillin showed the highest antimicrobial activity with an MIC of 0.63 mg/ml against *R. solanacearum*. Against *P. capsici*, *m*-cresol, *p*-cresol, *p*-ethylphenol, and 3,5-xylenol showed strong antimicrobial activity (MIC of 0.31 mg/ml). With regard to *F. oxysporm*, 3,5-xylenol showed very strong activity with an MIC of 0.16 mg/ml, but *p*-cresol had an MIC of 0.31 mg/ml, and *m*-cresol and *p*-ethylphenol had an MIC of 0.63 mg/ml. As for the activity against *P. splendens*, *p*-ethylphenol showed very strong activity with an MIC of 0.16 mg/ml, but phenol, pyrocatechol, and 3,5-xylenol had an MIC of 0.31 mg/ml, and cresols had an MIC of 0.63 mg/ml. Syringols had almost the same antimicrobial effect as that of the guaiacols, but were not contained in the *C. japonica* wood vinegar. Some phenolic compounds such as phenol and cresols have been well known as an antimicrobial agent and the relationship between the structures and antimicrobial activities of substituted phenols were already reported [22, 23]. Both the phenols and the guaiacols may considerably contribute to the antimicrobial activities of the wood vinegar, because the total content of the phenols and the guaiacols was about 0.32 mg/ml at the MIC (20 mg/ml) of the wood vinegar against *F. oxysporum* and *P. splendens*.

The antimicrobial effects of the furans and cyclotene were much weaker than that of the phenols and guaiacols. Maltol showed relatively strong activity, having an MIC of 0.63 mg/ml and 1.25 mg/ml against *R. solanacearum* and *P. splendens*, respectively, but was weak against *P. capsici*.

Thus, since both the phenols and the guaiacols are contained in the wood vinegar at relatively high concentration (total content 15.6 mg/ml) and have significantly higher antimicrobial activity than the other types of constituents, they are regarded as major antimicrobial constituents in the *C. japonica* wood vinegar.

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