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NOTE

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Inhibition of the harmful alga *Microcystis aeruginosa* by sugi (*Cryptomeria japonica*) bark

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Abstract

To utilize woody waste for the inhibition of harmful algae, we examined the growth inhibitory activities of sugi (*Cryptomeria japonica*) bark powder against *Microcystis aeruginosa*, which is a cyanobacteria species that causes algal blooms in lake or pond areas. The growth of *M. aeruginosa* was inhibited by the direct mixing of sugi bark powder into media in flasks. The inhibitory activity against *M. aeruginosa* was decreased when the bark underwent organic solvent extractions. Thus, the inhibitory activity was promoted by the extract components. After solvent extraction, the bark maintained a level of inhibitory activity suggestive of both chemical and physical effects. These results show that the growth of *M. aeruginosa* can be suppressed by directly using sugi bark with no extraction treatment.

Keywords: Woody wastes, Extract, Algal bloom, Allelopathy, Lake environment

Introduction

Applications of tree components with allelopathic activities have been studied as methods to suppress algal blooms, which present an environmental problem when caused by harmful algae, such as cyanobacteria species, in lake or pond areas [1, 2]. Nakai et al. [3] reported the inhibition activity of phenolic compounds such as catechin of plant components against the cyanobacterial sp. *Microcystis aeruginosa*. Tsuchiya et al. [4] studied the inhibitory effects of water extracts from woody plant leaves on *M. aeruginosa* growth, and found that extracts with higher polyphenol contents tended to show higher inhibitory effects. In our recent study [5], bark and heart wood extracts of sugi (*Cryptomeria japonica*) showed growth inhibitory activities against *M. aeruginosa*. However, there are factors that limit their practical applications, such as extraction costs when using an organic solvent and the environmental risks of using chemicals.

Sugi is a major Japanese forest product and its wood is abundantly used for house building and pulping materials. Sugi bark and wood mill residue, regarded as woody waste products, are generated in large quantities from the wood industry so effective uses for these are required [6, 7]. Traditionally, sugi logs, as a timber material, were often stored in water in a process known as pond or water storage in Japan, and this method has also been useful in timber production [8, 9]. Thus, sugi wood and bark materials are considered ecologically safe in a water environment compared with other plant components or chemical reagents. Although the addition of high concentrations of sugi bark extracts to the lake is not practical in terms of impact on other organisms and cost, it is considered relatively safe and cost-effective to use the bark directly without pretreatment. Therefore, we examined the ability of sugi bark powder to inhibit the growth of *M. aeruginosa* to determine the feasibility of its practical applications. The effects of extracted compounds were also examined by comparing the activities of bark samples before and after being subjected to the extraction process.

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Materials and methods

Extraction and preparation of bark powder

Sugi bark samples from Nagasaki Prefecture were supplied by the Kyushu Regional Agricultural Administration Office of Japan. A section of whole bark was first separated into outer and inner bark, then the bark was cut into fragments of less than 1 cm using scissors. The bark samples were successively exposed to hexane, ethyl acetate, and methanol to obtain separate extracts. Flavanol contents of ethyl acetate and methanol extracts obtained from whole bark were determined by the vanillin–HCl method [10] using catechin as a standard. The residual bark after extraction was ground using a Wiley mill to produce the extracted bark powder. Non-extracted bark powder was prepared using a Wiley mill before the extraction process.

Table 1 Yields and growth inhibitory activities of successive extracts obtained from sugi bark

	Extraction solvent	Yield[a] (%)	Inhibitory activity[b] (% ± SE)
Inner bark	Hexane	12.1	23.2 ± 1.09
	Ethyl acetate	5.48	62.5 ± 1.08
	Methanol	12.4	63.2 ± 0.459
Outer bark	Hexane	5.47	48.2 ± 0.790
	Ethyl acetate	1.61	38.1 ± 1.69
	Methanol	1.21	41.5 ± 1.85
Whole bark	Hexane	8.42	27.6 ± 1.30
	Ethyl acetate	3.14	51.7 ± 0.498
	Methanol	5.75	50.7 ± 1.07

SE standard error

[a] Yield shows percentage of each extract based on dry weight of bark material

[b] Bioassay of each extract were conducted at 5 mg/mL of concentration. Inhibitory activities (IA) were calculated by following equations.
IA = 100 × (1-cell density in each sample test/cell density in control test)

Bioassay

Bioassays to determine the inhibitory effects of bark extracts against *M. aeruginosa* strain NIES-87 (obtained from the National Institute for Environmental Studies) were performed using 5.0 μg/mL initial extract concentrations in 5 mL of *M. aeruginosa* medium (MA medium) as described previously [5]. In the assay using bark powder, *M. aeruginosa* was inoculated into 100 mL of MA medium at *ca.* 8×10^5 cell/mL initial cell density in a 200-mL Erlenmeyer flask. The bark powder was directly mixed at concentrations of 1.0, 5.0, 10, and 50 mg per 100 mL of medium. The test period was 14 days, and the number of cells was measured with a Thomas hemocytometer every 48 h. The level of growth inhibition was calculated using the inhibition percentage based on the algal growth of the control as described in a previous report [5].

Results and discussion

Activities of extracts

The yields and growth inhibitory activities of the successive extracts are shown in Table 1. The bark sample from Nagasaki prefecture contained high amounts of resin in the inner bark. Yields of bark extracts from this study were much higher than those reported previously [5]. When inner and outer bark were separated from whole bark (non-separated bark), their weights were almost the same, and the yield of each extract from whole bark was an intermediate value between the yields of the respective inner and outer bark extracts. The inhibitory activities of the inner and outer bark extracts were similar to those of a previous report [5]. The activity of each extract obtained from whole bark was intermediate between the activities of the respective inner and outer bark extracts. Ethyl acetate and methanol extracts had higher levels of activity than hexane extract in whole bark as well as inner bark. Active

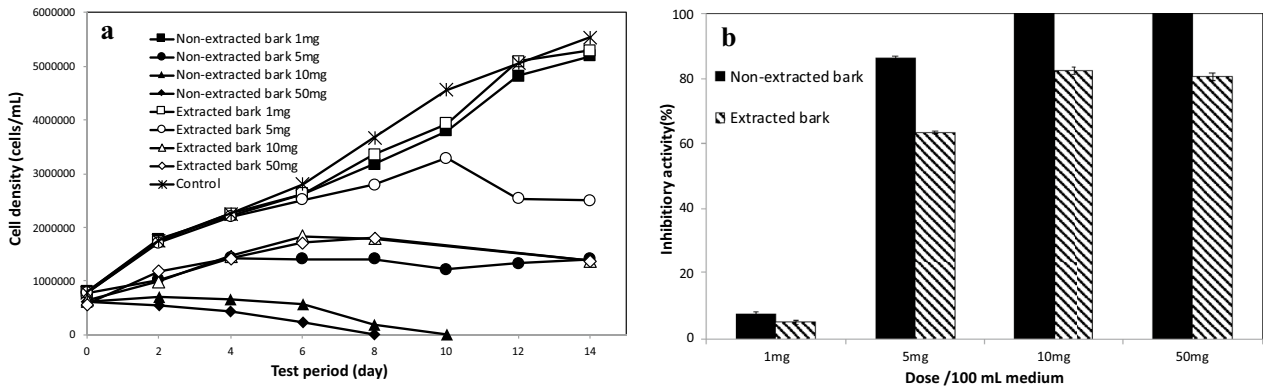
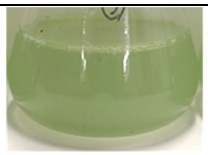
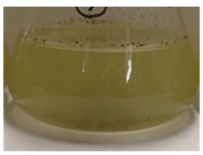
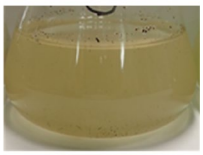
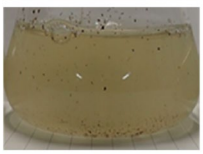
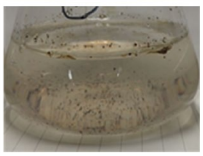
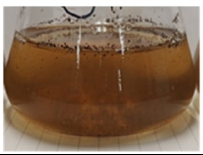
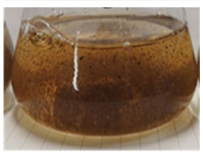
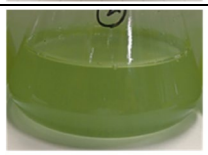
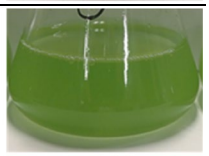
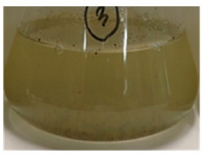
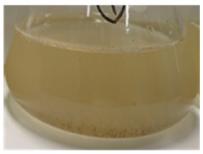
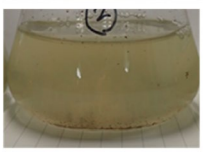
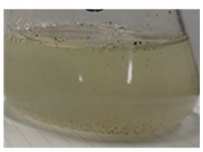
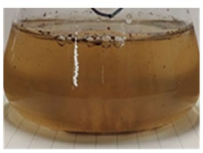
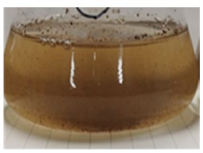
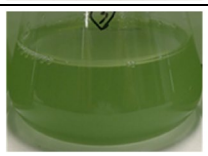
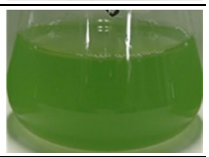


Fig. 1 Growth curve of *M. aeruginosa* during the 14-day bioassay (**a**). The inhibitory activity of bark powder compared with control after the bioassay (**b**). Bar: standard error (± SE)

compounds in ethyl acetate and methanol extracts of the inner bark were found to be flavanol compounds, as previously indicated [5], constituting 20.3% and 32.1% of the extract types, respectively. Flavanol compounds contained in the inner bark were the main active compounds of whole bark extracts.

Table 2 Photographs of culture media at 8 days and 14 days from the start of the bioassay

	Bark powder (Dose/100 mL)	Test Period (day)	
		8	14
Non-extracted bark	1 mg		
	5 mg		
	10 mg		
	50 mg		
Extracted bark	1 mg		
	5 mg		
	10 mg		
	50 mg		
Control	-		

Activities of bark powder

The algal growth curve and the inhibitory activity of bark powder against *M. aeruginosa* during the 14-day testing period are shown in Fig. 1a, b. In media injected independently with 1.0 mg/100 mL of non-extracted and extracted bark powders, the cell density transitions were similar to that of the control, and the inhibitory activity of non-extracted bark was greater than that of extracted bark. The inhibitory activity of the 5 mg/mL concentration of non-extracted bark was almost the same as that of 10 and 50 mg/100 mL concentrations of extracted bark. Remarkable inhibitory activities against *M. aeruginosa* were observed using non-extracted bark at 50 and 10 mg/100 mL concentrations. The cell density of the medium after the addition of 50-mg/100 mL non-extracted bark powder decreased immediately, and by day 8, floating algae could not be observed in the medium. Similarly, when 10-mg/100 mL non-extracted bark was added to the medium, the cell density decreased from day 6 to 10, and floating algae could not be observed by day 10.

Table 2 shows the color change of each culture medium during the test period. Culture media mixed with 10-mg/100 mL non-extracted bark became almost colorless by the end of the test. Thus, the growth of *M. aeruginosa* was inhibited by directly mixing bark powder into the culture medium, although the level of inhibitory activity varied greatly depending on the presence of components extracted by organic solvents. Bark extract components were required for strong inhibitory activity against *M. aeruginosa*; however, extracted bark powder also showed inhibitory activities at high concentrations. In the 50 mg/100 mL concentration test, the culture media of both non-extracted and extracted bark were brown in color (Table 2). We did not perform water extraction of bark samples after organic solvent extraction in this study. Because the color change of the culture media of extracted bark was caused by the water-soluble extract, we speculate that components not extractable by organic solvents, such as highly polar polyphenols, would also have activity. We also observed the adsorption of *M. aeruginosa* cells to the surface of both non-extracted and extracted bark powder microscopically, which suggested that the bark inhibited the growth of *M. aeruginosa* by a physical adsorption effect. This is a topic for future study to clarify the details of the water-soluble extract activity and the physical adsorption effect of sugi bark against *M. aeruginosa*.

Conclusion

The growth of *M. aeruginosa* was inhibited by the direct mixing of sugi bark powder with culture media. The inhibitory activity was greatly reduced in extracted bark powder, which indicates that the presence of extract components influences inhibitory activity. Because the extracted bark powder maintained an inhibitory activity, components in organic solvent extract residues may also show weak inhibitory activities that could be associated with physical adsorption. This study suggested that the addition of cedar bark directly to a lake or pond area may suppress the growth of *M. aeruginosa*, and proposed a new method of using sugi bark. For practical applications, it will be necessary to carry out safety experiments against other organisms and field tests in the future.

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Authors' contributions

YS, TJ, HS, KT, and TA conceived and designed the experiments. TJ collected sugi bark samples. YS performed the experiments. YS, HS, and TA analyzed experimental data and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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