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Screening method for wood extractives: direct cellulose thin-layer chromatography plate

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Abstract A method for screening wood extractives was developed using cellulose thin-layer chromatography plate (Cell-TLC) separation and was directly applied to bioassays. Wood meal (<60 mesh) from nangka (*Artocarpus heterophyllus* Lamk) heartwood was extracted with hot methanol. The crude extract was separated using a Cell-TLC plate (50 × 50 mm). Two broad bands with R_f values of 0.46 and 0.96 were found, and the bands showed completely different effects against the pest termite *Coptotermes formosanus* Shiraki and the decay fungus *Fomitopsis palustris*. The band with R_f 0.46 was preferentially consumed by workers of *C. formosanus*, and it did not show any growth inhibition against *F. palustris* when the Cell-TLC plate was directly exposed to the organism. In contrast, the band with R_f 0.96 appeared to repel strongly the feeding by *C. formosanus* and inhibited the growth of *F. palustris*. It was concluded that the Cell-TLC system was applicable for screening wood extracts consisting of many compounds.

Key words Screening test · Wood extractives · Cellulose-TLC plate · *Coptotermes formosanus* · *Fomitopsis palustris*

Introduction

Most methods for evaluating the biological durability of wood have focused on termites and decay fungi as deteriorating organisms. It is well known that one of the most important factors affecting the biological characteristics of wood is the extract. Bioassays for evaluating the biological activities of wood extract have been mainly conducted with treated filter papers,[1–14] paper disks,[15–18] cellulose paper pads,[19–22] and wood powders[9–14,23] for termites or extract-containing agar media for decay fungi.[24,25] Using these methods, the separation of crude extracts and the bioassays are time-consuming because numerous samples or chromatographic fractions are evaluated against target organisms.

Thin-layer chromatography (TLC) is useful for separating mixtures of organic compounds. It is also expected to be applicable for separating crude extracts of wood consisting of numerous compounds. Silica gel, cellulose, and other materials are used as stationary phases for TLC; and the crude extract on a cellulose-TLC (Cell-TLC) plate would be separated on the plate and directly applied to bioassays, as termites and decay fungi can use the cellulose layer in the Cell-TLC plate as their carbon source.

Escoubas et al.[26] applied TLC plates for the separation of insect antifeedant compounds from extracts of *Skimmia japonica* Thunb. together with the direct bioassay using larvae of *Spodoptera litura* (Fabricius). For the bioassay, they poured specified nutrients on the silica gel surfaces of TLC plates as the diet and finally obtained three antifeedant compounds: bergaptan, xanthotoxin, and oxypeucedanin. For wood-deteriorating organisms, only Minato et al.[24] reported direct use of the Cell-TLC system to identify antifeedant compounds from the crude extract of *Bagassa quianensis* Aubl. against the pest termite *Coptotermes formosanus* Shiraki, although conventional agar media were used for the bioassay with decay fungi. It can be said that no TLC-based screening method applicable to bioassays in both termites and decay fungi has yet been developed. A novel method for screening wood extracts against termites and decay fungi is described herein.

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Materials and methods

Wood extract

Wood meals (<60 mesh) from the heartwood of nangka (*Artocarpus heterophyllus* Lamk) more than 20 years old

Table 1. Pretreatment on Cell-TLC medium combinations

Method	Pretreatment on the Cell-TLC	Medium
I	–	Fungus-growth agar
II	Fungal inoculation[a]	Agar
III	Distilled water[b]	Fungus-growth agar
IV	Distilled water + fungal inoculation	Agar
V	Agar medium[c]	Fungus-growth agar
VI	Agar medium + fungal inoculation	Agar
VII	Liquid medium[d]	Fungus-growth agar
VIII	Liquid medium + fungal inoculation	Agar

Cell-TLC, cellulose thin-layer chromatography
[a] Cell-TLC was in contact with the surface of the fungus-growth agar medium
[b] Cell-TLC was superficially in contact with distilled and sterilized water
[c] Cell-TLC was superficially in contact with a hot agar medium contained 3.9 g potato agar in 100 ml distilled water
[d] Cell-TLC was superficially in contact with a liquid medium contained 4 g glucose, 0.3 g peptone, and 1.5 g malt extract in 100 ml distilled water

were air-dried to approximately 15% moisture content and extracted with hot methanol at refluxing temperature for 20 min. The crude extract was used for the TLC separation and bioassays.

Cellulose-TLC separation

Cell-TLC without a fluorescence indicator (Merck, 50 × 50 mm) was used for separation of the crude extract and subsequent bioassays. The crude extract was applied to the bottom of the Cell-TLC plate and was developed with 2-butanol/acetic acid/water (14:1:5, v/v).[24,27] The compounds were visualized by spraying a 0.2% (w/v) solution of 2′,7′-dichlorofluorescence followed by exposure to ultraviolet-254 (UV_{254}). The locations of the compounds on the Cell-TLC plate were defined by the R_f value, which is defined as the distance of the spot center from the starting point divided by the distance of the solvent front from the starting point.[28]

Feeding test with termites

Sandy soil 30 g (<20 mesh) and 5 ml of distilled water were placed in a petri dish (90 mm diameter, 20 mm height). The Cell-TLC with the separated crude extract was put on the soil with 200 *C. formosanus* workers collected from a laboratory colony maintained in the Wood Research Institute, Kyoto University at 28° ± 2°C and >85% relative humidity (RH) in the dark. The assembled petri dish was kept in the dark at 28° ± 2°C and >85% RH for 8 days. Termite feeding on the Cell-TLC plate was evaluated daily. A control and a blank were employed for the experiment: The control was an undeveloped Cell-TLC plate without the extract; the blank was a developed Cell-TLC plate without the extract. Three replicates were used for each test.

Growth inhibition test with decay fungi

Fomitopsis palustris (Berk. Et. Curt) FFPRI–0507 and *Trametes versicolor* (L. Ex. Fr) Quel FFPRI-1030, the test fungi, were inoculated on the Cell-TLC plate. Before evaluating the samples, various pretreatments, media, or both were examined for the amount of fungal growth on the Cell-TLC plates. Table 1 shows the pretreatment–medium combinations. Based on the results of the preliminary trials, method VIII was selected as the test method for evaluating the effect of the extract on Cell-TLC plates against *F. palustris*. The controls were the same as for the termite feeding test.

Results and discussion

Cellulose-TLC separation

The hot methanol crude extract of nangka heartwood was yellow, and the amount was 10.36% (w/w). After separation, two broad bands with R_f values of 0.46 and 0.96 were found when developed until the end of Cell-TLC. The band with R_f 0.46 was not colored, whereas the band with R_f 0.96 was yellow.

Feeding test with termite

Results of the termite feeding test are shown in Fig. 1. In the control and blank, *C. formosanus* workers evenly consumed the cellulose layer, and there was no cellulose remaining on the Cell-TLC plate after 8 days of exposure. In the sample, *C. formosanus* workers preferentially consumed the band with R_f 0.46 at the early stage of the test. Thereafter, the insects consumed the entire surface of the Cell-TLC plate except for the band with R_f 0.96, which was intact even after an 8-day exposure. These results suggest that components in the bands with R_f 0.46 and 0.96 act as attractants and repellents, respectively, against *C. formosanus* workers.

Growth inhibition test with decay fungi

The results of the preliminary trial are shown in Table 2. All the methods could be used to inoculate *F. palustris* on the

Table 2. Growth of *Fomitopsis palustris* and *Trametes vesicolor* on the Cell-TLC plate after 9 days (first trial)

Method	*F. palustris*	*T. versicolor*
I	+	−
II	+	+
III	+	+
IV	+	−
V	+	+
VI	+	+
VII	+	−
VIII	+	+

+, growth; −, no growth

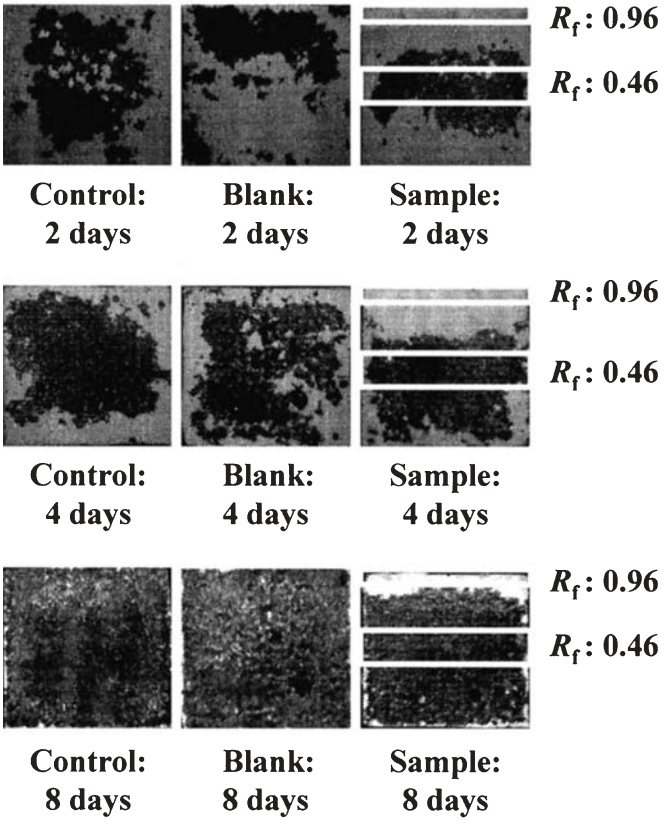

Fig. 1. Results of the feeding test of *Coptotermes formosanus* workers on a cellulose thin-layer chromatography (Cell-TLC) plate with the separated crude hot methanol extract of nangka heartwood. Cellulose layers were consumed by termites in the *black areas* but not in the *white areas*. *Control*, undeveloped Cell-TLC without nangka extract; *Blank*, developed Cell-TLC without nangka extract; *Sample*, developed Cell-TLC with nangka extract

Cell-TLC plate, whereas *T. versicolor* did not grow on the Cell-TLC prepared using methods I, IV, or VII. After observing the growth activity of *F. palustris* and *T. versicolor*, methods II, III, and VIII were chosen for the second trial, where repeatability was evaluated. Methods V and VI were not used for the second trial because of the difficulty preparing the Cell-TLC with agar media.

The results of the second trial are shown in Table 3. The growth of *F. palustris* was more extensive than that of *T. versicolor*, and the repeatability of methods II and VIII was

Table 3. Growth of *F. palustris* and *T. vesicolor* on the Cell-TLC plate after 9 days (second trial)

Method	*F. palustris*			*T. versicolor*		
	1	2	3	1	2	3
II	++	++	++	+	−	+
III	−	−	−	−	−	−
VIII	++	++	++	+	+	+

++, good growth; +, poor growth; −, no growth

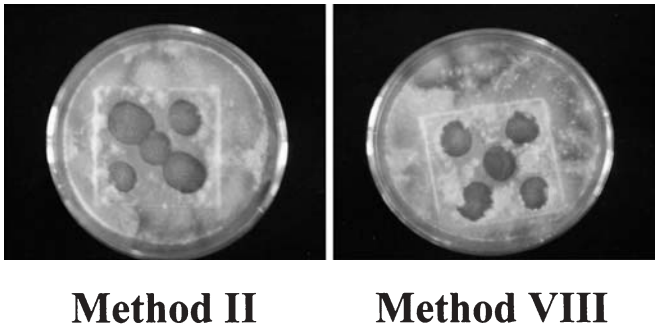

Fig. 2. Growth of *Fomitopsis palustris* on Cell-TLC with some drops of hot methanol crude extract of nangka heartwood with methods II and VIII after a 9-day exposure (third trial)

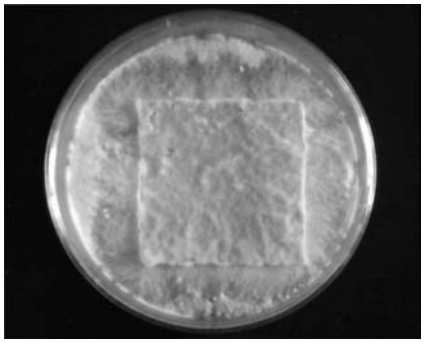

Fig. 3. Growth of *F. palustris* on a Cell-TLC plate with the separated hot methanol crude extract of nangka heartwood using method VIII after a 9-day exposure. There was no fungal growth observed in the band with an R_f value of 0.96

higher than that of method III. Therefore, for the third trial (the last preliminary trial) the Cell-TLC plate with some drops of the crude nangka extract was exposed to *F. palustris* using methods II and VIII.

As shown in Fig. 2, after a 9-day exposure *F. palustris* showed more growth with method VIII than with method II, characteristically around the sample drops. Based on the results of the third trial, method VIII was chosen for sample testing.

Fomitopsis palustris grew evenly on the surface of the Cell-TLC plate in the control and the blank, but in the sample the fungus did not grow in the band with R_f 0.96 (Fig. 3). This result clearly suggests that method VIII is

applicable as a direct antifungal screening test following TLC separation of wood extract. Moreover, the band with R_f 0.96 might be a mixture of components, as it effectively inhibited fungal growth and repelled *C. formosanus* workers.

Conclusions

The Cell-TLC plate with separated hot methanol crude extract of nangka (*Artocarpus heterophyllus* Lamk) heartwood was directly applied to bioassays with termites and decay-inducing fungi. Termite feeding behavior on the Cell-TLC plate clearly indicated the characteristics of two bands: one with an attractant and the other with a repellent. Repeated trials resulted in developing a novel method for evaluating growth inhibition of the bands separated by the Cell-TLC system. Separation of the crude wood extract and evaluation of its biological characteristics can be conducted rapidly when applying this method.

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