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Short note

Isolation and identification of antifungal compounds from Amboyna wood

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Introduction

In continuation of our research into biologically active compounds from trees and their production by tissue culture (Tachibana and Sumimoto 1989; Tachibana et al. 1994; Miyata et al. 1998; Muranaka et al. 1998; Premjet et al. 2002; Yoshida et al. 2002), antifungal compounds were isolated from Amboyna wood.

Plants are a rich source of chemical substances, with over 100,000 plant products described to date (Vicente et al. 2003), and play an important role in the production of biologically active secondary metabolites. Many secondary metabolites such as flavonoids, stilbenes, lignans and sesquiterpenoids have antifungal activities (Reyes-Chilpa et al. 1998; Celimene et al. 1999; Chang et al. 2000; Kawamura et al. 2004).

Amboyna, *Pterocarpus indicus* Willd. (Leguminosae), is an important tropical tree, known as angsana and sena in Malaysia and Indonesia or narra in the Philippines, which mainly grows in Southeast Asia. Its natural distribution seems to cover the southern part of Burma, Java and New Guinea (Ogata 1985). Amboyna wood has been used for high-quality furniture and cabinets, decorative veneers, interior wall paneling, boat building and specialized joinery (Eddowes 1977). Extracts of leaves, stem and bark of Amboyna were reported to be active against several bacteria and protozoan (Khan and Omoloso 2003), while a polyphenolic compound isolated from young bark was reported to be a plasmin activity inhibitor (Takeuchi et al. 1986).

In the present paper, we report the ability of Amboyna wood to inhibit the growth of wood-rotting fungi and the isolation of active compounds that play a role in the defense system of the wood.

Materials and methods

The experimental instruments were the same as described in our previous report (Kusuma et al. 2004). Authentic samples of isoliquiritigenin and liquiritigenin were synthesized from the reaction of resacetophenone (2',4'-dihydroxyacetophenone) and *p*-hydroxybenzaldehyde by aldol condensation (Nadkarni and Wheeler 1938; Kurth 1939). Acetylation of the compounds isolated was conducted with pyridine and acetic anhydride in the usual way.

Wood samples

Amboyna wood samples were collected in Indonesia in 2001 and sawn into lumbers. The lumbers were converted into woodmeal with a Wiley Mill. Woodmeals were passed through #40 mesh and were kept in the laboratory after their moisture content was measured.

Antifungal assay

Antifungal activity of the wood and wood extracts against a white-rot fungus, *Pleurotus pulmonarius*, which is an important wood-rotting fungus of commercial hardwood timber in Indonesia, was evaluated using the agar dilution method. Potato dextrose agar (PDA) (20 mL) and 2 g of woodmeal or the respective amount of extract sample dissolved in acetone were mixed on a 90-mm Petri dish and sterilized. Details of the antifungal assay method are described in our previous report (Kusuma et al. 2004). Antifungal activity was determined based on inhibition using the formula: percentage inhibition = $(1 - T/C) \times 100$, where T is the hyphal extension of the treated sample and C is the hyphal extension of the control (culture medium and acetone). The mean value from triplicate experiments of each plate was used for statistical analysis, and the significance of differences was determined using Student's t-test.

Extraction and isolation of compounds 1, 2 and 3

Amboyna woodmeal (1.2 kg) was extracted twice with methanol for 8 h at 65 °C and the methanol solution obtained was evaporated on a rotary evaporator to give methanolic extracts. The methanolic extracts were suspended in water and extracted with *n*-hexane, diethyl ether, ethyl acetate and *n*-butanol, respectively. A portion (8 g) of the diethyl ether solubles (D, 62 g), one of the active solubles along with the *n*-hexane solubles, was separated by silica gel column chromatography with $CHCl_3$–MeOH (2%–50% MeOH), which resulted in six fractions [(D.1)–(D.6)]. Antifungal assays of the fractions showed that (D.2) (0.7 g) and (D.3) (2.5 g) were most active, causing 91% inhibition. Fraction (D.3) (720 mg) was re-chromatographed over silica gel and eluted with $CHCl_3$–MeOH (0.5%–50% MeOH) and a further eight fractions were collected [(D.3.1)–(D.3.8)]. Fractions (D.3.4) (440 mg) and (D.3.6) (88 mg) inhibited *P. pulmonarius* growth by 52% and 38%, respectively. Further separation of fraction (D.3.4) using silica gel column chromatography with $CHCl_3$–MeOH (1%–50% MeOH) gave six fractions [(D.3.4.1)–(D.3.4.6)]. Fractions (D.3.4.4) (116 mg) and (D.3.4.6)