Jurnal Manajemen Hutan Tropika Vol. XII No. 2 : 78-83 (2006) **Artikel (*Article*)**

ANTIFUNGAL ACTIVITIES OF THE EXTRACTS FROM SOME TROPICAL AND TEMPERATE WOODS

(Aktivitas Antifungi Ekstrak Beberapa Kayu Daerah Tropis dan Iklim Sedang)

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ABSTRAK

Empat flavonoids (Sciadopitysin) (1), Ginkgetin (2), Ishorhamnetin (3), dan Quercetin (4), dua Texanes (Taxinine) (5) dan Taxol (6), dan Phenylisoserine methyl ester (7) dapat diisolasi dari Taxus cuspadata var. nana. Senyawa (7) juga dapat diisolasi dari Taxus chinensis. Senyawa (7) dapat diisolasi untuk pertama kalinya masing-masing dari T. cuspidata var. nana dan T. chinensis.Aktivitas antifungus senyawa-senyawa yang terisolasi tersebut dan tujuh turunan taxinine terhadap tujuh fungi patogenik tanaman (Cochliobolus miyabeanus, Alternaria kikuchiana, Giberella fujikuroi, Cladosporium cucumenium, Fusarium oxysporum, Colletotrichum fragariae dan Corynespora cassiicola) telah diukur. Taxinine dan tujuh turunan taxinine menunjukkan adanya aktivitas antifungus terhadap lima jenis fungi. Diantara tiga jenis kayu tropis (Amboyna,Kempas,Angsana),kayu Amboyna menunjukkan aktivitas antifungus terhadap empat fungi dari basidiomycetes (Le,Pp,G3,dan PLI).Selanjutnya,ekstrak methanolic dan fraksi larutnya n-hexane dan ethyl ether dari kayu Amboyna menunjukkan aktivitas antifungus terhadap fungus Pp.

Kata kunci: Antifungi, flavonoid, Texanes, phenylisoserine methyl ester, kayu tropis dan temperate, fungi patogenik tanaman, basidiomycetes.

INTRODUCTION

Tropical as well as temperate woods contain many biologically active compounds with antifungal, antibacterial, anticanker activities, hypotensive effect and so. However, there are not so many woods of which the active constituents have been examined. Though the actions of artificial antifungal agents to harmful insects and fungi are strong, there is a problem in the safety for the organisms. Furthermore, they remain in the long time in the environment. However, it is said that antifungal agents deriving from natural products are safe to the organism and degrade it by microorganism. Therefore, antifungal activities of the compounds existed in *Taxus chinensis* and *T. cuspidata var. nana* trees against some

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plant pathogenic fungi were examined. And furthermore, antifungal activities of three kinds of tropical woods against four kinds of basidiomycetes fungi were examined.

METHODS

Extraction of Taxus cuspidata var. nana leaves

The Leaves of *T. cuspidata var. nana* (600g) were extracted with methanol twice at room temperature. The methanolic extracts were suspended with water and successively extracted with *n*-hexane, cloroform, ethyl acetate and *n*-butanol, respectively. The chloroform and ethyl acetate soluble fractions were combined and subjected to silica gel column chromatography. Four kinds of flavonoids and two kinds of terpenoids were isolated from the fractions. And, phenylisoserine methyl ester was isolated from the ethyl acetate soluble fraction. In addition, phenylisoserine methyl ester was synthesized by the method of Guo *et al.* (1993).

Extraction of *Taxus chinensis* bark

The bark of *T. chinensis* was extracted with ethanol twice at room temperature. The ethanol extracts were suspended with water and successively extracted with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol, respectively. Phenylisoserine methyl ester was isolated from the ethyl acetate soluble fraction.

Extraction of four kinds of tropical woods

Four kinds of tropical woods were extracted with methanol to give each methanolic extracts. The extracts with antifungal activities were further separated in the same manner as described above.

Antifungal active test

Fungi used for the tests: Seven plant pathogenic fungi (*Cochliobolus miyabeanus, Alternaria kikuchiana, Gibberella fujikuroi, Cladosporium cucumenium, Fusarium oxysporum, Colletotrichum fragariae,* and *Corynespore cassiicola*) and four basidiomycetes fungi (fungi Le, Pp, G3,and PL1) were cultured in the potato-dextrose-agar (PDA) medium. Antifungal active test against the basidiomycetes fungi was conducted by the method of Nakajima *et al.* (1980). In addition, the onion-dextrose agar medium was used for antifungal active test against the basidiomycetes. Antifungal active test against the plant pathogenic fungi were carried out on the (PDA) medium by reference to the method of Muranaka *et al.* (1997).

RESULTS AND DISCUSSION

Isolation of the compounds from *T. cuspidata*

Six kinds of compounds, Sciadopitysin (1), Ginkgetin (2), Isorhamnetin (3), Quercetin (4), taxinine (5) and taxol (6) were isolated from the chloroform and ethyl acetate soluble fractions in the yield of 0.0039, 0.0018, 0.0024, 0.015, 0.029 and 0.0002%, respectively to the fresh leaves, and phenylisoserine methyl ester was isolated from the ethyl acetate soluble fraction in the yield of 0.0003% to the fresh leaves[6], The compound (7) was identified as phenylisoserine methyl ester by comparison of the NMR and Mass spectra with authentic sample synthesized by the method of Gou *et al.* (1993). It is thought that the compound (7) is an artifact produced during the extraction of *T. cuspidata* leaves with methanol. However, the compound (7) was also isolated when extraction solvent was changed from methanol to ethanol. Therefore, the compound (7) was not artifact. Phenylisoserine methyl ester (7) was isolated for the first time from *T. cuspidata var. nana.* Chemical structures of compounds 1-7 are shown in Fig. 1.

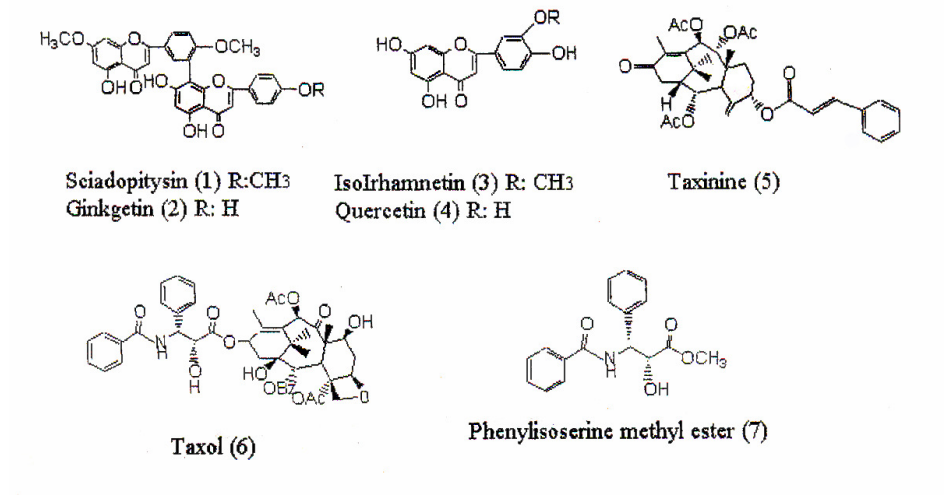

Figure 1. Chemical structures of compounds 1-7.

Isolation of phenylisoserine methyl ester from the bark of *T. chinensis*

Phenylisoserine methyl ester (7) was also isolated for the first time from the ethyl acetate soluble fraction in the ethanolic extract from *T. chinensis*. Existence of the compound (7) is not unclear. However, the compound (7) is the side chain of taxol. Therefore, the compound (7) may be incorporated into biosynthetic route of taxol [7,8] after hydrolysis of the compound (7) with an esterase in the both taxus trees.

Antifungal activities of flavonoids and taxanes against *C. miyabeanus* and *A. kikuchiana*

The results are shown in Table 1 and 2. Sciadopitysin (1), Quercetin (4) and taxinine (5) showed antifungal activity against *C. miyabeanus*. To find more active compounds than taxinine, seven taxinine derivatives were prepared from taxinine, and their antifungal activities against two the plant pathogenic fungi were measured. Among the seven derivatives, bisdeacetyltaxinine, isopropylidenebisdeacetyl taxinine, isopropyridenedeacetyl taxinine, and taxinine epoxide showed almost the sameactivities as that of taxanine againt *C. miyabeanus*. On the contrary, antifungal activities of the five compounds (1-5) and seven taxinine derivatives against A. kikuchiana were weak, and the activities were almost one-half of those against *C. miyabeanus*.

Table. 1 Minimum inhibitory concentration of compounds 1-5 against *Cochiobolus miyabeanus* and *Alternaria kikuchiana*

Compound	Minimal inhibitory concentration (μ mol)	
	C. miyabeanus	*A. kikuchiana*
1	0.2	1.7
2	0.9	1.8
3	1.6	1.6
4	0.4	3.3
5	0.2	1.7

Antifungal activities of flavonoids (1-4), taxanes (5 and 6), and phenylisoserine methyl ester (7) against the five fungi

Antifungal activities of four flaponoids (sciadopitysin (1), ginkgetin (2), isorhamnetin (3), and quercetin (4), two taxanes (taxinine (5) and taxol (6)), and phenylisoserin methyl ester (7) against the five plant pathogenic fungi were measured. The results were were shown in Table 2. As shown in the Table, taxanine methyl ester did not show the activities against the five fungi.

Table 2. Minimum inhibitory concentration of taxanine against the five used for the test

Test fungi	Minimal inhibitory concentration (μ mol)
Giberella fujikuroi	0.4
Cladosporium cucumenium	0.4
Fusarium oxysporum	0.4
Collectotrichum fragariae	-
Corynespore cassiicola	0.4

Antifungal activities of three kinds of tropical woods against the four fungi

The results are shown in Table 3. As shown in the table, Amboyna wood showed highest antifungal activities against the four kinds of basidiomycetes fungi. It has not been reported until now that Amboyna wood showed antifungal activities against the four fungi. To find antifungal compounds in Amboyna wood, the wood was extracted with methanol. The methanolic extracts were separated into *n*-hexane, ethyl ether, ethyl acetate, and *n*-butanol soluble fractions, respectively by liquid- liquid extraction as described above. Antifungal activities of the methanolic extracts and its each soluble fraction against the four fungi were measured. As shown in Table 4, *n*-hexane and ethyl ether soluble fractions showed antifungal activities against the fungus Pp. at present, antifungal compounds in these fractions are being conducted.

Table 3. Antifungal activities of three Indonesian woods

Test fungi	Antifungal activities		
	Amboyna	Kempas	Angsana
Le	+ + +	+	+
Pp	+ + +	-	-
G3	+ +	-	-
PLI	+ +	-	-

Notes: + + +: strong; + +; medium; +: weak. Le: *Lentinula edodes* (from East Java);
Pp: *Pleurotus pulmonarius* (from West Java); G3 : *Ganoderma* sp. (from Japan);
PLI: *Pleurotus ostreatus* (from Japan).

Table 4. Antifungal activities of the methanolic extracts and its each Soluble fraction from Amboyna woods against fungus Pp

Fractions	Inhibiton (%)
Methanolic extracts	100
n-Hexane soluble fractions	35
Ethyl ether soluble fraction	46
Ethyl acetate soluble fractions	0
n-Butanol soluble fraction	0

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