ANTIFUNGAL ACTIVITIES OF EXTRACTS FROM HEARTWOOD, SAPWOOD AND BARK OF 11 MALAYSIAN TIMBERS AGAINST *GLOEOPHYLLUM TRABEUM* AND *PYCNOPORUS SANGUINEUS*

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KAWAMURA F, MAHAMUD A, SULAIMAN O & HASHIM R. 2010. Antifungal activities of extracts from heartwood, sapwood and bark of 11 Malaysian timbers against *Gloeophyllum trabeum* and *Pycnoporus sanguineus*. Antifungal activities of 33 methanol extracts obtained from the bark, sapwood and heartwood of 11 Malaysian timbers, *Dipterocarpus apterus*, *Shorea curtisii*, *Hopea odorata*, *Calophyllum rubiginosum*, *Calophyllum symingtonianum*, *Cynometra inaequifolia*, *Swintonia schwenkii*, *Dyera costulata*, *Sandoricum koetjape*, *Pimeleodendron griffithianum* and *Pterocarpus indicus* were evaluated against the brown-rot fungus *Gloeophyllum trabeum* and the white-rot fungus *Pycnoporus sanguineus* using a medium in which homogenised hyphae were dispersed. The heartwood of *C. symingtonianum* and the outer wood of *S. schwenkii* showed high antifungal activities against *G. trabeum* while the heartwoods of *P. indicus* and *C. symingtonianum*, the bark and sapwood of *P. griffithianum*, and the sapwood of *C. rubiginosum* showed high antifungal activities against *P. sanguineus*. The activities of methanol extracts from selected parts of these wood species were higher than that of the positive control, glycyrrhizic acid dipotassium salt, and results suggest the potential of these extracts as fungistats.

Keywords: Methanol extracts, brown-rot fungus, white-rot fungus, fungistat

KAWAMURA F, MAHAMUD A, SULAIMAN O & HASHIM R. 2010. Aktiviti antikulat ekstrak kayu teras, kayu gubal dan kulit kayu 11 spesies dari Malaysia terhadap *Gloeophyllum trabeum* dan *Pycnoporus sanguineus*. Aktiviti antikulat 33 ekstrak metanol diperoleh daripada kayu teras, kayu gubal dan kulit kayu 11 spesies pokok dari Malaysia iaitu *Dipterocarpus apterus*, *Shorea curtisii*, *Hopea odorata*, *Calophyllum rubiginosum*, *Calophyllum symingtonianum*, *Cynometra inaequifolia*, *Swintonia schwenkii*, *Dyera costulata*, *Sandoricum koetjape*, *Pimeleodendron griffithianum* dan *Pterocarpus indicus*. Ekstrak ini diuji terhadap kulat reput perang (*Gloeophyllum trabeum*) dan kulat reput putih (*Pycnoporus sanguineus*) menggunakan medium yang mempunyai hifa homogen. Kayu teras *C. symingtonianum* dan kayu luar *S. schwenkii* menunjukkan aktiviti antikulat yang tinggi menentang *G. trabeum* sementara kayu teras *P. indicus* dan *C. symingtonianum*, kulit kayu serta kayu gubal *P. griffithianum* dan kayu gubal *C. rubiginosum* menunjukkan aktiviti antikulat yang tinggi terhadap *P. sanguineus*. Aktiviti ekstrak metanol daripada bahagian tertentu spesies kayu ini lebih tinggi daripada kawalan positif iaitu garam asid glisirizik dikalium. Keputusan mencadangkan bahawa ekstrak ini berpotensi dijadikan fungistat.

INTRODUCTION

Plants contain a huge variety of secondary metabolites to protect themselves from diseases and harsh environments such as plant pathogen and ultraviolet light. Over 4000 different flavonoids have been isolated from plants, mainly from foliage, bark, sapwood and heartwood of trees (Obst 1998), and many of them provide resistance to fungi and insects (Harborne 1989). Terpenoids represent the largest class of secondary metabolites and are derived from isoprene (isopentane) C_5 building blocks. Terpenoids are found throughout nature and occur in almost all plants. In fact, they have been exploited since antiquity as perfumes, insect repellents, fungicides and medicines (Obst 1998). Some of the other secondary metabolites include phenolic acid, phenolic aldehyde, saponins, stilbenoids, lignans, fatty acid, xanthones and coumarins.

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Many approaches are used to control the endless variety and complexity of fungal diseases (Agrios 2009, Lee *et al.* 2009). Many secondary metabolites of timber have antifungal properties (Quiroga *et al.* 2001, Carpinella *et al.* 2003, Kawamura *et al.* 2004, Kawamura & Ohara 2005, Kusuma *et al.* 2005, Yen *et al.* 2007) and can be used as natural biodegradable fungicides to replace the traditional toxic wood preservatives, which create environmental hazards (Carpinella *et al.* 2003, Yen *et al.* 2007).

In this study, a total of 33 extracts obtained from bark, sapwood and heartwood of 11 selected Malaysian commercial timbers were evaluated for their antifungal activities against the white-rot fungus *Pycnoporus sanguineus* and the brown-rot fungus, *Gloeophyllum trabeum*. The standard antifungal assay using spores cannot be applied to *G. trabeum* and *P. sanguineus* because these fungi do not form basidiospores unless they form mycelia. Therefore, in the present study, antifungal assays of *G. trabeum* and *P. sanguineus* using homogenised hyphae were used.

Licorice (*Glycyrrhiza glabra*) is rich in bioactivities such as antiviral, anticancer, antiulcer, antidiabetic, anti-inflammatory, antioxidant, antithrombic, antimalarial, antifungal, antibacterial, estrogenic, immuno stimulant, antiallergenic and expectorant activities. Its major secondary metabolite, glycyrrhizic acid dipotassium salt (GADS), is utilised for antifungal/antibacterial clothes (Rastogi & Mehrotra 1989). Therefore, GADS was used as positive control in the present study.

MATERIALS AND METHODS

Plant materials

Samples of 11 species of Malaysian timbers were obtained from sawmills in Kedah and Penang, Malaysia, namely, (common local name (common English name, *botanical name*, family)), keruing latek (keruing, *Dipterocarpus apterus,* Dipterocarpaceae), meranti seraya (dark red meranti, *Shorea curtisii*, Dipterocarpaceae), merawan siput jantan (merawan, *Hopea odorata,* Dipterocarpaceae), bintangor daun karat (bintangor, *Calophyllum rubiginosum,* Clusiaceae), bintangor bukit (bintangor, *Calophyllum symingtonianum,* Clusiaceae), kekatong (kekatong, *Cynometra inaequifolia*, Leguminosae), merpauh periang (merpauh, *Swintonia schwenkii,* Anacardiaceae), jelutong (jelutong, *Dyera costulata,* Apocynaceae), sentul (santol, *Sandoricum koetjape,* Meliaceae), perah ikan (pimeleodendron, *Pimeleodendron griffithianum,* Euphorbiaceae), and angsana (angsana, amboyna wood, *Pterocarpus indicus,* Fabaceae). The timbers were identified by the Kedah Forestry Department. A disc was prepared from each log which was then separated into bark, heartwood and sapwood and ground to < 1 mm in a Wiley mill (Retsch, SM 1). Heartwood and sapwood were discerned by the naked eye. Samples that had no clear demarcation between heartwood and sapwood were separated into either inner wood (within the point of 25% radius from the centre) or outer wood (beyond the point of 80% radius from the centre). Voucher specimens were deposited at the Division of Bio-resource, Paper and Coatings Technology, Universiti Sains Malaysia.

Extraction

Each air-dried sample meal (1 g) was extracted under reflux with 70 ml methanol for 6 hours. Another batch of air-dried sample meals (each sample 0.7 g) was ovendried at 105 °C for 16 hours and their moisture contents were calculated. The extracted solution was filtered and the solvent was removed *in vacuo* (30 °C) in a rotary evaporator. The yield (%) of methanol extracts was calculated based on oven-dry weights of the samples.

Antifungal assay

Antifungal assays were performed following methods described in previous papers (Kawamura *et al.* 2004, Kawamura & Ohara 2005). The fungal strains used were *G. trabeum* MI-102 obtained from the School of Biology, Universiti Sains Malaysia and *P. sanguineus* KUM 70097, from the Forest Research Institute Malaysia (FRIM). The fungi were incubated for 10 days in a liquid malt extract medium. After incubation hyphae were homogenised for 2 min at 10 000 rpm. Subsequently, the liquid medium was removed by centrifugation and the homogenised hyphae were washed with physiological saline. The hyphae (1 ml) were added to 12 ml sterilised potato dextrose agar medium and mixed using a glass rod for 5 s. The mixture was then poured into 9-cm Petri dishes. Using micropipetts, sterilised paper discs (diameter 6 mm, Advantec Toyo Inc) were permeated with 10 µl of the methanol

solutions (2.5, 5, 10, 20, 50, 100 µg/µl) containing each of the methanol extracts or the positive control GADS. The discs were allowed to dry at room temperature for 15 min. The discs with extract concentration of 25, 50, 100, 200, 500 or 1000 µg/disc were then placed on the agar surface in each dish. The width of the inhibition zone around each disc was measured after three days of incubation at 26 °C and recorded as ++ (for inhibition zone diameter > 10 mm), + (7–10 mm) or - (no inhibition zone). Tests were carried out in triplicates.

RESULTS AND DISCUSSION

The fungal growth inhibitory activities and yields of the 33 methanol extracts samples are summarised in Table 1. The heartwood and sapwood of *H. odorata* and bark of *C. symingtonianum* and *P. indicus* showed very high yields of methanol extracts. However, except for the bark of *P. indicus*, these samples showed no or very weak antifungal activities against *G. trabeum* and *P. sanguineus*. The extracts of these species appeared to contain large amounts of less active antifungal constituents. In addition, methanol extracts from bark or heartwood of many of the wood species had higher yields than those from sapwood. This shows that the concentration of secondary metabolites in trees is not uniform; generally higher amounts occur in bark, heartwood, roots, branch bases and wound tissues (Obst 1998).

The heartwood of *C. symingtonianum* showed the highest antifungal activity against *G. trabeum* followed by the outer wood of *S. schwenkii*, both with minimum inhibition concentration of only 50 µg/disc. The activities of these two extract samples were higher than those of positive control GADS. The inner wood of *S. schwenkii* showed moderate antifungal activity against *G. trabeum*. However, all bark samples showed no activity against *G. trabeum*. The sapwood and bark of *P. griffithianum* showed the highest antifungal activity against *P. sanguineus*, requiring less than 25 µg/disc to cause inhibition. The heartwoods of *P. indicus* and *C. symingtonianum*, and the sapwood of *C. rubiginisum* also showed high antifungal activities against *P. sanguineus*. Activities of these extracts were higher than those of GADS at the five concentrations tested. All parts of *S. curtisii* and *D. costulata* and the bark of *P. indicus* showed moderate antifungal activities against *P. sanguineus*. The antifungal minimum inhibition concentrations of extract samples against *P. sanguineus* in the present study were less than those reported for extracts of *Anacardium occidentale* against *P. sanguineus* (Adetogun & Adegeye 2003). Although extracts from bark was not effective against *G. trabeum*, bark of several wood species showed antifungal activity against *P. sanguineus*. In general, heartwood showed higher durability than sapwood because of the higher amount of extracts in the former compared with the latter (Obst 1998). However, in the present study, the outer wood of *S. schwenkii* and the sapwoods of *P. griffithianum* and *C. rubiginosum* also showed exceptionally high activities. Results suggest the potential of these parts of wood species to be used as plant material for extraction of fungistats.

Unlike stilbenoids which only showed high activity against brown-rot fungi *G. trabeum* and *Poria placenta* and not against the white-rot fungus *Coriolus versicolor* (Schultz *et al.* 1991), results of the present experiment proved that methanol extract from the heartwood of *C. symingtonianum* had strong antifungal activity against brown- and white-rot fungi. *Calophyllum inophyllum* is known to show various bioactivities and there are many reports of isolation of xanthones from this species (Spino *et al.* 1998, Dharmaratne *et al.* 1999, Itoigawa *et al.* 2001, Yimdjo *et al.* 2004). Belonging to the same genus, we believe *C. symingtonianum* in this study had high antifungal activity due to presence of xanthones.

CONCLUSIONS

Antifungal activities of extracts obtained from certain parts of the wood species tested were higher than those of the positive control GADS. This suggests that these extracts can be used as fungistats. These extracts showed high activities without any fractionation or purification. Therefore, they have an advantage in cost to produce the fungistats. However, these extracts must be tested for the treatment of wood in order to prove its effectiveness in the improvement of decay durability.

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Table 1 Antifungal activities of methanol extracts and their yields

Species	Part	Fungal strain												Yield (%)
		Gloeophyllum trabeum						*Pycnoporus sanguineus*						
		Concentration of extracts (µg/disc)						Concentration of extracts (µg/disc)						
		1000	500	200	100	50	25	1000	500	200	100	50	25	
Pterocarpus indicus	B	-	-	-	-	-	-	+	+	+	+	-	-	14.53
	H	+	-	-	-	-	-	+	+	+	+	+	+	5.68
	S	+	-	-	-	-	-	-	-	-	-	-	-	7.23
Pimeleodendron griffithianum	B	-	-	-	-	-	-	++	+	+	+	+	+	3.51
	H	-	-	-	-	-	-	+	-	-	-	-	-	5.13
	S	-	-	-	-	-	-	++	++	+	+	+	+	1.67
Calophyllum symingtonianum	B	-	-	-	-	-	-	-	-	-	-	-	-	16.10
	H	++	++	++	++	++	-	+	+	+	+	+	+	3.13
	S	-	-	-	-	-	-	-	-	-	-	-	-	4.49
Calophyllum rubiginisum	B	-	-	-	-	-	-	+	-	-	-	-	-	6.09
	H	-	-	-	-	-	-	-	-	-	-	-	-	3.63
	S	+	-	-	-	-	-	++	+	+	+	+	-	1.63
Swintonia schwenkii	B	-	-	-	-	-	-	-	-	-	-	-	-	7.70
	I	++	+	+	-	-	-	-	-	-	-	-	-	2.69
	O	++	+	+	+	+	-	-	-	-	-	-	-	3.63
Sandoricum koetjape	B	-	-	-	-	-	-	-	-	-	-	-	-	9.14
	I	-	-	-	-	-	-	-	-	-	-	-	-	8.34
	O	-	-	-	-	-	-	-	-	-	-	-	-	7.52
Shorea curtisii	B	-	-	-	-	-	-	++	+	+	-	-	-	3.51
	I	-	-	-	-	-	-	++	++	+	+	-	-	3.70
	O	-	-	-	-	-	-	++	+	+	+	-	-	2.31
Hopea odorata	B	-	-	-	-	-	-	++	-	-	-	-	-	8.65
	H	-	-	-	-	-	-	-	-	-	-	-	-	17.50
	S	++	-	-	-	-	-	++	-	-	-	-	-	13.10
Cynometra inaequifolia	B	-	-	-	-	-	-	-	-	-	-	-	-	5.55
	H	-	-	-	-	-	-	-	-	-	-	-	-	4.43
	S	-	-	-	-	-	-	-	-	-	-	-	-	4.90
Dyera costulata	B	-	-	-	-	-	-	++	+	+	+	-	-	6.15
	I	-	-	-	-	-	-	++	+	+	-	-	-	3.69
	O	-	-	-	-	-	-	++	+	+	-	-	-	3.67
Dipterocarpus apterus	B	-	-	-	-	-	-	-	-	-	-	-	-	5.77
	H	-	-	-	-	-	-	-	-	-	-	-	-	3.18
	S	-	-	-	-	-	-	-	-	-	-	-	-	6.49
GADS		++	++	++	-	-	-	++	++	++	++	-	-	

++ = Inhibition zone > 10 mm diameter; + = inhibition zone < 10 mm diameter; - = no inhibition zone; B = bark; H = heartwood; S = sapwood; I = inner wood; O = outer wood; GADS = glycyrrhizic acid dipotassium salt

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