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Evaluation on Antioxidant Activity, Antifungal Activity and Total Phenols of 11 Selected Commercial Malaysian Timber Species

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Abstract

Antioxidant activity [1,1-diphenyl-2-picrylhydrazyl (DPPH) assay], antifungal activity against *Gloeophyllum trabeum* (brown-rot) and *Pycnoporus sanguineus* (white-rot), and total phenol content (Folin-Ciocalteu method) of 11 selected commercial Malaysian timbers were investigated. The extracts from Neem bark showed the highest yield, 25.59%. Kelat gelam bark showed the highest antioxidant activity, followed by Kelat jambu air bark. The extracts that showed the four highest antioxidant activities were all taken from bark samples. These extracts also showed high yields of methanol extracts and high total phenol content, suggesting that they have great potential as a source of antioxidant material. The highest total phenol content was found in Neem bark, while the lowest was in Ramin melawis bark. The methanol extracts from the heartwood of Neem showed the highest antifungal activity against *G. trabeum*. The methanol extracts from the sapwood and the heartwood of Neem, and the heartwood of Kulim showed the highest antifungal activity against *P. sanguineus*. The antifungal activities of these methanol extracts were higher than those of the positive control, glycyrrhizic acid dipotassium salt. Almost all wood species showed antifungal activity against either brown- or white-rot fungus. However, methanol extracts from the heartwood of Neem showed strong antifungal activity against brown- and white-rot fungi, *G. trabeum* and *P. sanguineus*, suggesting that they have great potential as a source of fungistats.

Discipline: Forestry and forest products

Additional key words: basidiomycete, 1,1-diphenyl-2-picrylhydrazyl, Folin-Ciocalteu method, *Gloeophyllum trabeum*, *Pycnoporus sanguineus*

Introduction

Currently there are about 100 commercial timber species in Malaysia. These commercial species are used for making sawlogs, sawn timber, plywood, veneer, moldings, furniture, and so on. The chemical components of these timber species are also important and need to be exploited. In general, extracts from bark and heartwood have strong biological activities such as antioxidant[8] and antifungal[7, 12] activities in tree species. Wood extractives represent a number of different compounds that can be extracted from wood by means of polar and non-polar solvents. Several woods contain extractable substances that are deterrents to bacteria, fungi and termites, while other extractives give color and odor to woods[1, 17].

Antioxidant refers to any substance that hinders the reaction of a substance with dioxygen or any substance that inhibits free radical reaction[3]. Antioxidants have gained more importance on account of their positive effects as health promoters in the treatment of cardiovascular problems, atherosclerosis, many forms of cancer,

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the ageing process, etc., and many antioxidant compounds naturally occurring in plant sources have been identified as free radical scavengers[2, 10].

Antifungal refers to a chemical compound produced biosynthetically or synthetically that could destroy or usefully suppress the metabolism of a variety of harmful microscopic organisms. Only a small number of these microscopic agents, however, are safe chemotherapeutic agents, effective in controlling infectious disease in "plants, animals and man"[16]. Antimicrobial agents contain various functional groups. However, there is a particular structural type that seems to favor antimicrobial activity[21].

Plant pathogens including fungi, nematodes, bacteria, and viruses can cause disease or damage in plants[14]. Plant pathogens, particularly fungi, are responsible for yield reductions in crops throughout the world. Biodegradation of wood by fungi is a serious problem for both wood structure and forest management. Despite the fact that many plants are affected by fungal disease, some of them are able to manufacture their own antifungal compounds[13]. Brown-rot and white-rot, which are discussed in the present paper, and soft-rot are important types of wood decay, and have been thoroughly investigated. Brown-rot fungi attack soft wood and are responsible for the most serious types of decay in wooden structures[22].

Phenolic acids are phenolic compounds that have an aromatic ring to which a carboxylic acid group is attached. They are common in plants either free or combined into esters or glycosides. Many phenolic acids are antibacterial and antifungal[20]. Phenolic compounds including simple phenols and phenolic acids, hydroxycinnamic acids derivatives and flavonoids are bioactive substances occurring widely in plants. Many phenolic compounds in plants are good sources of natural antioxidants. According to Pratt and Hudson (1992)[15], phenolic compounds are abundant in all parts of plants, such as wood, bark, stems, leaves, fruit, roots, flowers, pollen and seeds.

In this paper, antioxidant activity, antifungal activities against *Gloeophyllum trabeum* (a brown-rot fungus) and *Pycnoporus sanguineus* (a white-rot fungus), and total phenols of selected commercial Malaysian timbers were investigated. The potential of extracts from commercial Malaysian timbers as a source of antioxidant and fungistats were evaluated.

Materials and methods

1. Plant materials

The following 11 species of commercial Malaysian timbers cultivated in Kedah, Malaysia, were sampled in 2007: Kelat jambu air (*Syzygium samarangense*, Myrtaceae), Kelat gelam (*Eugenia Cerina*, Myrtaceae), Kelat merah (*Eugenia Chorantha*, Myrtaceae), Kulim (*Scorodocarpus Horneensis*, Olacaceae), Penarahan arang (*Myristica Cinnamomea*, Myristicaceae), Terap nasi (*Artocarpus Elasticus*, Moraceae), Kasai daun besar (*Pometia Pinnata*, Sapindaceae), Nyatoh tembaga kuning (*Palaquium Hispidum*, Sapotaceae), Rhu (*Casuarina Equisetifolia*, Casuarinaceae), Neem (*Azadirachta Indica*, Meliaceae), and Ramin melawis (*Gonystylus Bancanus*, Thymelaeaceae). The samples were separated into three parts; bark, heartwood and sapwood, and ground in a Wiley mill (Retsch, cutting mill SM 1) into wood meal less than 1 mm in diameter, then air-dried. For samples with no clear determination of sapwood and heartwood, they were separated as inner wood and outer wood.

2. Extraction

Each air-dried sample meals (1 g, equivalence oven-dried weight, 0.85-0.92 g) was extracted under reflux with 70 mL methanol for 6 h. The other air-dried sample meals (0.7 g) was oven-dried, and moisture contents of sample meals 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-dried plant material weight.

3. Antioxidant activity (DPPH radical scavenging assay)

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the tested samples was measured according to the previously described method with a slight modification[9]. Briefly, the absorbance at 517 nm was recorded 30 min after preparation of test solutions. The percentage of DPPH scavenging activity was determined by

$$A = [(A_0 - A_e)/A_0] \, 100$$

where, A represents the percentage reduction of the DPPH, A_0 is the initial or blank solution absorbance, and A_e is the absorbance value for a sample concentration in the absence of DPPH solution. This activity was also expressed as the 50% inhibition concentration in the final tested solution (EC_{50}). EC_{50} values based on the final concentration of extracts in test solution were determined[5]. (+)-Catechin was used as a positive control. Tests were carried out in triplicate.

4. Antifungal assay

Antifungal assays were performed based on our

previous paper[11]. The fungal strains used were *Gloeophyllum trabeum* (L.: Fr.) Murr. MI-102 from the School of Biology, Universiti Sains Malaysia (USM) and *Pycnoporus sanguineus* (Pers.: Fr.) Murr. KUM 70097 from the Forest Research Institute Malaysia (FRIM). PDA medium was mixed with homogenized hyphae, and poured into the petri dishes. Paper discs (diameter 6 mm, Advantec Toyo Inc.) were permeated with 10 µl of the acetone or MeOH solutions (5, 10, 20, 50, 100 µg/µl) containing each MeOH extracts. A paper disc permeated with glycyrrhizic acid dipotassium salt (GADS) and a disc without constituents were used as positive and negative controls, respectively. The paper disc was placed into the medium that contained potatoes glucose agar (PDA) and homogenized hyphae. The dishes were incubated in the dark at 26°C and 70% relative humidity for 3 days, and their respective inhibition zones were observed. The results of the antifungal assay were expressed as follows: when the diameter (mm) of the inhibition zone was > 15, there was very clear inhibition (+++); at 15 >, > 10, there was clear inhibition (++); and at < 10, there was partial inhibition (+). When the inhibition zone was the same as the negative control or there was no inhibition zone, it was considered as no inhibition (−).

5. Total phenols assay (Folin-Ciocalteu method)

Total phenol content of the extract was determined using the Folin-Ciocalteu method with a slight modification[19]. A 10% Folin-Ciocalteu reagent (2.5 ml) was mixed into 500 µl of a 120 µg/ml sample solution. After 3 min, 2.0 ml 7.5% sodium carbonate aqueous solution was added, and the mixture was allowed to stand for 2 h. Absorbance readings were taken at 740 nm. The concentration of total phenols was determined as a percentage of equivalent with an equation obtained from the (+)-catechin calibration curve, based on linear regression: $TP = [((A - y) / z) / w] \times 100$, where TP is the total phenols content (%), A is the absorbance, y and z are the equivalents found from the calibration curve and w is the weight of samples (µg). Tests were carried out in triplicate.

Results and discussion

Yield, antioxidant activity (EC_{50} value) and total phenols of methanol extracts from bark, heartwood and sapwood of 11 Malaysian timber species are shown in Table 1. The extracts from Neem bark showed the highest yield, 25.59%. Kelat gelam bark showed the highest antioxidant activity (EC_{50}, 3.00±0.58 µg/ml), followed by Kelat jambu air bark (EC_{50}, 3.57±1.24 µg/ml). The extracts that showed the four highest antioxidant activities were all taken from bark samples. These extracts also showed high yields of methanol extracts and high total phenol content, suggesting that they have great potential as a source of antioxidant material.

The total phenol content varied greatly among samples, with the highest total phenols found in Neem bark (69.14%), while the lowest was in Ramin melawis bark (5.60%). The correlation between antioxidant activity (data of the samples that showed EC_{50} less than 30 µg/ml was plotted) and total phenol content is shown in Fig. 1. Antioxidant activity is expressed as antiradical power ($1/EC_{50}$)[5]. There was some correlation between antioxidant activity and total phenol content. However, methanol extracts from Rhu inner wood and Kasai daun besar heartwood did not show antioxidant activity at 30 µg/ml, although total phenol contents in these extracts were relatively high. The results in Fig. 1 suggest the contribution of phenolic constituents to antioxidant activity. However, the amount of phenolic constituents may not be the only factor involved in antioxidant activity and the molecular structures of different phenolic constituents may play an important role in antioxidant activity[3].

The results of the antifungal assay against a brown-rot fungus, *Gloeophyllum trabeum* and a white-rot fungus, *Pycnoporus sanguineus* are summarized in Table 2. Fourteen parts from eight species showed the antifungal activity against *P. sanguineus*, although only four parts from four species showed the antifungal activity against *G. trabeum*. The methanol extracts from the heartwood of Neem showed the highest antifungal activity against *G. trabeum*, followed by methanol extracts from the heartwood of Nyatoh tembaga kuning. The minimum effective amounts of the methanol extracts from Neem heartwood and Nyatoh tembaga kuning heartwood were 50 µg and 100 µg, respectively, and they were less than the minimum effective amount (200 µg) of the positive control, glycyrrhizic acid dipotassium salt (GADS). The methanol extracts from the sapwood and the heartwood of Neem, and the heartwood of Kulim showed the highest antifungal activity against *P. sanguineus*. The minimum effective amounts of the methanol extracts from the sapwood and the heartwood of Neem, and the heartwood of Kulim, were all less than 25 µg, and they were much less than the minimum effective amount (100 µg) of GADS. The results of the present experiment indicated that most wood species showed antifungal activity against either brown- or white-rot fungus. However, methanol extracts from the heartwood of Neem showed strong antifungal activity against brown- and white-rot fungi, *G. trabeum* and *P. sanguineus*. Neem is well

Table 1. Yield (%), antioxidant activity (EC_{50}) and total phenols (%) of methanol extracts from bark, heartwood, and sapwood of commercial Malaysian timber species

Species	Parts[a]	Yield[b] (%)	Antioxidant activity[c] EC_{50}(μg/ml)	Total phenols[d] (%)
Kelat jambu air *(Syzygium samarangense)*	Bark	15.80	3.57±1.24	52.45±6.52
	Heartwood	16.11	8.50±2.12	29.02±4.33
	Sapwood	8.94	9.07±3.33	27.15±4.16
Ramin melawis *(Gonystylus bancanus)*	Bark	18.48	4.29±0.90	60.35±6.84
	Inner wood	21.21	>30	27.56±3.99
	Outer wood	4.30	>30	22.14±3.18
Penarahan arang *(Myristica cinnamomea)*	Bark	10.12	8.29±0.25	32.32±6.29
	Inner wood	12.08	5.29±0.65	51.28±1.17
	Outer wood	9.91	5.46±1.04	49.15±1.84
Rhu *(Casuarina equisetifolia)*	Bark	18.48	4.29±0.90	60.35±6.84
	Inner wood	3.50	>30	45.20±4.76
	Outer wood	2.70	9.93±0.10	36.87±3.87
Kasai daun besar *(Pometia pinnata)*	Bark	4.19	14.43±1.82	28.93±2.64
	Heartwood	14.04	>30	39.53±2.49
	Sapwood	2.90	28.57±0.34	24.51±2.49
Kelat gelam *(Eugenia cerina)*	Bark	13.87	3.00±0.58	57.28±7.99
	Heartwood	13.19	11.14±2.02	23.51±1.80
	Sapwood	7.92	8.19±0.66	22.60±0.75
Kelat merah *(Eugenia chorantha)*	Bark	2.95	8.67±0.08	36.15±2.79
	Heartwood	5.52	24.64±1.52	15.42±2.07
	Sapwood	2.08	>30	9.40±1.19
Kulim *(Scorodocarpus horneensis)*	Bark	2.83	>30	14.52±0.83
	Heartwood	1.56	>30	16.57±2.18
	Sapwood	1.88	>30	13.04±1.77
Nyatoh tembaga kuning *(Palaquium hispidum)*	Bark	10.30	9.64±1.31	37.09±0.86
	Heartwood	9.24	27.86±2.53	24.48±0.04
	Sapwood	4.73	23.81±2.18	31.73±3.99
Terap nasi *(Artocarpus elasticus)*	Bark	5.30	>30	11.89±1.53
	Heartwood	8.69	>30	21.25±2.31
	Sapwood	2.21	>30	14.80±1.90
Neem *(Azadirachta indica)*	Bark	25.59	4.00±0.14	69.14±9.97
	Heartwood	8.32	7.95±0.50	50.69±5.10
	Sapwood	2.53	18.57±2.02	22.98±5.70
(+)-Catechin[e]			3.43±0.44	

[a] : Outer wood and inner wood were separated from samples that have no clear demarcation between sapwood and heartwood
[b] : Percentage based on the weight of oven dried plant materials
[c] : EC_{50} values based on final concentration of extract in test solution
[d] : Percentage based on the weight of methanol extracts
[e] : Positive control
Result are represented as means ± standard deviation (n=3)

Table 2. Antifungal activities[a] of the methanol extracts from commercial Malaysian timber species against *Gleophyllum trabeum* and *Pycnoporus sanguineus*

Species	Parts	Inhibition zone											
		Gleophyllum trabeum						*Pycnoporus sanguineus*					
		I	II	III	IV	V	VI	I	II	III	IV	V	VI
Kelat jambu air	Bark	−	−	−	−	−	−	−	−	−	−	−	−
	Heartwood	−	−	−	−	−	−	−	−	−	−	−	−
	Sapwood	−	−	−	−	−	−	−	−	−	−	−	−
Ramin melawis	Bark	−	−	−	−	−	−	+++	++	++	++	−	−
	Inner wood	−	−	−	−	−	−	−	−	−	−	−	−
	Outer wood	−	−	−	−	−	−	−	−	−	−	−	−
Penarahan arang	Bark	−	−	−	−	−	−	−	−	−	−	−	−
	Inner wood	−	−	−	−	−	−	−	−	−	−	−	−
	Outer wood	−	−	−	−	−	−	−	−	−	−	−	−
Rhu	Bark	−	−	−	−	−	−	−	−	−	−	−	−
	Inner wood	++	+	−	−	−	−	++	−	−	−	−	−
	Outer wood	−	−	−	−	−	−	−	−	−	−	−	−
Kasai daun besar	Bark	−	−	−	−	−	−	++	−	−	−	−	−
	Heartwood	++	+	+	−	−	−	++	−	−	−	−	−
	Sapwood	−	−	−	−	−	−	+++	++	−	−	−	−
Kelat gelam	Bark	−	−	−	−	−	−	−	−	−	−	−	−
	Heartwood	−	−	−	−	−	−	−	−	−	−	−	−
	Sapwood	−	−	−	−	−	−	−	−	−	−	−	−
Kelat merah	Bark	−	−	−	−	−	−	+++	+++	++	++	+	−
	Heartwood	−	−	−	−	−	−	−	−	−	−	−	−
	Sapwood	−	−	−	−	−	−	−	−	−	−	−	−
Kulim	Bark	−	−	−	−	−	−	++	+	−	−	−	−
	Heartwood	−	−	−	−	−	−	+++	+++	+++	++	++	+
	Sapwood	−	−	−	−	−	−	+++	−	−	−	−	−
Nyatoh tembaga	Bark	−	−	−	−	−	−	−	−	−	−	−	−
kuning	Heartwood	+++	++	+	+	−	−	+++	++	−	−	−	−
	Sapwood	−	−	−	−	−	−	−	−	−	−	−	−
Terap nasi	Bark	−	−	−	−	−	−	++	++	++	++	+	−
	Heartwood	−	−	−	−	−	−	−	−	−	−	−	−
	Sapwood	−	−	−	−	−	−	+++	+++	+++	−	−	−
Neem	Bark	−	−	−	−	−	−	−	−	−	−	−	−
	Heartwood	+++	+++	++	+	+	−	+++	++	+	+	+	+
	Sapwood	−	−	−	−	−	−	+++	+++	+++	++	++	+
GADS[b]		++	++	++	−	−	−	++	++	++	++	−	−
Paper disc[c]		−	−	−	−	−	−	−	−	−	−	−	−

[a] : Sample amounts in paper discs; I=1,000 μg, II=500 μg, III=200 μg, IV=100 μg, V=50 μg, VI=25 μg. Inhibition zones with a diameter (mm) of > 15 showed very clear inhibition (+++); diameters of 15 >, > 10 showed clear inhibition (++); and diameters of < 10 showed partial inhibition (+). Inhibition zones with a diameter that same as negative control or no inhibition zone were expressed as no inhibition (−). Tests were carried out in triplicate.

[b] : Positive control, glycyrrhizic acid dipotassium salt (GADS).

[c] : Negative control.

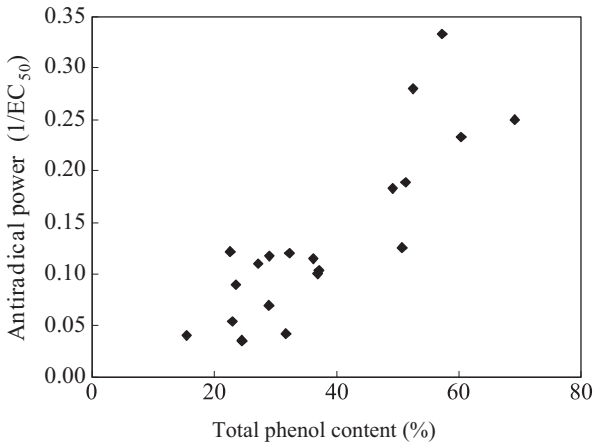

Fig. 1. The correlation between antioxidant activity of methanol extracts from Malaysian timbers and total phenol content in methanol extracts.
Antioxidant activity is expressed as antiradical power $(1/EC_{50})$[5].

known as a highly versatile medicinal plant having widespread traditional uses to cure various human diseases[4, 6, 18]. To our knowledge, however, there is no report on Neem wood which describes its utilization as folk medicine or biological activities. The results of the antifungal assay for the methanol extracts from the heartwood and sapwood of Neem suggest that they have great potential as a source of fungistats.

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