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Evaluation of biological activities of extracts from 22 African tropical wood species

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Abstract Heartwoods of 22 African tropical wood species were extracted with methanol and the contents of total phenolic compounds in these extracts were measured. Three bioassays were conducted to evaluate the antioxidant activity, tyrosinase inhibitory activity, and antifungal activity of the methanol extracts. The results indicated that the extracts from 13 species exhibited high antioxidant potential, and their inhibitory concentrations that caused 50% scavenging of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (IC_{50}) were less than 10 μg/ml. The crude extract from *Cylicodiscus gabunensis* showed the highest antioxidant activity, and was even higher than that of (+)-catechin, which is known as a potent antioxidant. There was a good correlation between the antioxidant activity and the content of total phenolic compounds, indicating that phenolic compounds played a predominant role in the antioxidant property of the wood extracts. Among all 22 species, only 1 species, *Milicia excelsa*, contained extractives that showed very high tyrosinase inhibitory activity. The extracts from 9 species showed high antifungal activity. No consistent relationship was observed between the tyrosinase inhibitory activity or antifungal activity and the content of total phenolics in the extracts.

Key words Antioxidant activity · Tyrosinase inhibitory activity · Antifungal activity · African tropical wood species · Total phenolic compounds

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Introduction

Some tropical wood species have high extractives content in the heartwood. In addition to contributing to wood color and odor, extractives have also been found to have specific biological activities, such as antioxidant activity, plant growth regulation effects, and antimicrobial activity.[1,2] African forests are the world's second largest tropical reservoir and contain high levels of biodiversity. Tropical African tree species are very promising plant materials in the search for natural products with various biological activities because the plants grow under very severe conditions such as strong ultraviolet rays and high temperatures. Therefore, African plants, especially African medicinal plants, have attracted considerable research interest.[3–5]

In this study, we chose 22 African tropical hardwood species, 16 from Ghana and 6 from Tanzania, and evaluated their biological activities. Some of these wood species from different African countries have been investigated for the extractives in their barks or heartwood because of their various medicinal functions.[6–9] However, the biological activities of the extracts from the heartwood of these wood species from Ghana or Tanzania have not been reported before. For these reasons, three bioassays were selected to evaluate the antioxidant activity, tyrosinase inhibitory activity, and antifungal activity of the heartwood extracts of the 22 African tropical hardwood species. The results will provide useful information for screening species with heartwood extracts that possess very strong biological activities. This will allow further exploitation of new natural resources for various applications in improving human health.

Cutaneous hyperpigmentation, including freckles, skin stains, and senile lentigines, is a common pigmentary disorder in humans that becomes more prominent with age,

especially in Asians. To date, most research on the regulation of melanogenesis has focused on the factors affecting tyrosinase. Tyrosinase, also known as polyphenol oxidase (PPO), is a copper-containing, mixed-function oxidase that is widely distributed in microorganisms, animals, and plants. This oxidase catalyzes two distinct reactions of melanin synthesis, the hydroxylation of a monophenol and the conversion of an *o*-diphenol to the corresponding *o*-quinone. Kojic acid and arbutin are known tyrosinase inhibitors and are used as skin-whitening cosmetics.[10] In addition, tyrosinase is responsible for browning in plants and is considered to be deleterious to the color quality of plant-derived foods and beverages. This unfavorable darkening from enzymatic oxidation generally results in a loss of nutritional and economic value and is a cause of great concern.

In examining possible applications of wood extractives, the applications can be strongly linked to the activity of the extract. Specifically, the natural compounds with antioxidant activity could be used in health foods and beverages; compounds with tyrosinase inhibitory activities could be used as whitening agents and food additives; and the compounds with antifungal activity could be used for environmentally harmonized wood preservatives.

Materials and methods

Heartwood samples

Air-dried heartwood blocks of 22 tropical hardwood species (Table 1) were cut from freshly felled trees (one block with dimensions of 50 × 10 × 3 cm for each species) in Ghana or Tanzania, and delivered to Japan. The wood samples were resized into small blocks (10 × 10 × 10 mm) and ground into wood meals passing through a 0.5-mm screen in a Wiley Mill.

Preparation of methanol extracts

A portion of wood meal (2.5 g, moisture content ~10%) for each wood species was Soxhlet-extracted with methanol for 8 h. The methanol in the extraction solution was removed with a rotary evaporator. The residue was then dissolved or suspended in 10–20 ml of distilled water and lyophilized to give methanol extract powder. The extract yields were calculated as weight percentages of the oven-dried wood meals used for extraction.

Table 1. Extract yields, content of total phenolic compounds, antioxidant activity, tyrosinase inhibitory activity, and antifungal activity of methanol extracts from 22 African tropical hardwood species

Species	Common name	Extract yield (%)	Total phenolic compounds (mg/g)	IC_{50} of antioxidant activity[a] (μg/ml)	Relative tyrosinase activity[b] (%)	Minimum fungus inhibitory amount[c] (μg)
Afzelia africana	Papao	16.12	311	22.8	119.4	>200
Celtis zenkeri	–	3.04	137	26.4	95.1	20
Combretodendron macrocarpum	Esia	8.55	139	11.7	107.2	100
Commiphora mollis	–	17.75	379	8.2	85.8	50
Cylicodiscus gabunensis	Denya	15.27	868	1.4	104.0	>200
Entandrophragma cylindricum	Sapele	4.77	127	8.9	108.8	100
Gilbertiodendron spp.	Tetekon	1.75	138	15.7	113.2	10
Guarea cedrata	Guarea	7.05	93	17.2	111.0	>200
Heritiera utilis	Niangon	9.78	404	4.5	70.7	>200
Lophira alata	Ekki	1.69	129	16.1	95.6	50
Mammea africana	Bompagya	5.11	411	7.1	123.8	50
Manilkara discolor	–	1.80	181	20.6	126.4	20
Milicia excelsa	Odum	12.72	320	6.0	4.0	50
Nauclea diderrichii	Kusia	6.58	71	72.1	89.2	>200
Nesogordonia parviflora	–	3.80	245	6.3	100.1	>200
Nesogordonia papaverifera	Danta	7.66	326	5.0	91.8	>200
Piptadeniastrum africanum	Dahoma	5.34	310	6.9	96.7	50
Ptelopsis myrtifolia	–	2.58	390	4.2	94.7	20
Sterculia rhinopetala	Wawabima	2.18	100	18.7	95.4	>200
Terminalia ivorensis	Emire	18.21	607	2.5	76.9	>200
Tieghemella heckelii	Makore	12.32	210	7.8	92.4	>200
Uapaca kirkiana	–	8.94	227	6.4	92.9	200
(+)-Catechin[d]				2.1		
Kojic acid[e]					6.3	
Dimethyl sulfoxide[f]					100	
Hinokitiol[g]						10

[a] Oxidant concentration required for 50% scavenging of the initial 1,1-diphenyl-2-picrylhydrazyl radical
[b] Relative to activity in the presence of dimethyl sulfoxide
[c] Minimum sample amount required for the inhibition of fungal growth
[d] Positive control for antioxidant assay
[e] Positive control for tyrosinase inhibitory assay
[f] Control for tyrosinase inhibitory assay
[g] Positive control for antifungal assay

Total phenolic compounds in methanol extracts

Determination of total phenolic compounds in the methanol extracts was carried out based on the Folin–Ciocalteu method with slight modification.[11] Five milligrams of lyophilized extract powder was dissolved in 50 ml of methanol. A portion of this methanol solution (600 µl) was transferred to a 10-ml flask and evaporated to remove methanol. The residue in the flask was dissolved with 3 ml of water and the solution was quantitatively transferred to a 10-ml volumetric flask. One milliliter of Folin–Ciocalteu phenol reagent (Kanto Kagaku, Tokyo, Japan) was added, and the flask was shaken vigorously. Then 5 ml of 20% sodium carbonate aqueous solution was added, and the mixture was made up to 10 ml with water and shaken thoroughly again. After 20 min of incubation, the absorbance of the mixture was measured at 735 nm. The working curve was determined using catechin as a standard. The content of total phenolic compounds was expressed as the weight of total catechin equivalents in the lyophilized methanol extract (mg/g).

Evaluation of biological activities

Antioxidant assay

The antioxidant assay for scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was conducted according to the method described by Wang et al.[12] The reaction mixtures were prepared by mixing 1000 µl of 0.1 mM DPPH solution in ethanol, 450 µl of 0.05 M Tris-HCl buffer (pH 7.6), and 50 µl of test sample methanol solution (final concentrations were 1, 2, 3, 5, 10, 50, and 100 µg/ml, respectively) or methanol (used as control). Reduction of the DPPH free radical was measured by recording the absorbance at 517 nm exactly 30 min after each extract solution was added. (+)-Catechin was used as a positive reference in the test. The inhibition ratio was expressed as a percentage after being calculated from Eq. 1:

$$\%\ \text{Inhibition} = \left[\frac{(A_c - A_s)}{A_c}\right] \times 100 \quad (1)$$

where A_c is the absorbance of the control solution and A_s is the absorbance of the test sample solution.

The inhibitory concentration that caused 50% scavenging of the DPPH radical (IC_{50}) was estimated based on the plot of inhibition versus final concentration of the test samples. The relative antioxidant activity of the test sample in relation to (+)-catechin was calculated according to Eq. 2:

$$\%\ \text{Relative antioxidant activity} = \left[\frac{1/IC_{50_s}}{1/IC_{50_c}}\right] \times 100 \quad (2)$$

where IC_{50_s} is the IC_{50} value of the test sample and IC_{50_c} is the IC_{50} value of (+)-catechin.

Tyrosinase inhibitory assay

Tyrosinase activity was assayed by measuring the formation of 2-carboxy-2,3-dihydroindole-5,6-quinone (dopachrome) from 3,4-dihydroxy-L-phenylalanine (L-dopa) at pH 6.5 and 25°C.[13] L-Dopa solution (333 µl of 2.5 mM solution) was mixed with 600 µl of 0.1 M phosphate buffer ($Na_2HPO_4 \cdot 12H_2O/NaH_2PO_4 \cdot 2H_2O$) (pH 6.5) in a disposable ultraviolet/visible-grade cuvette, and incubated at 25°C. Thirty-three microliters of the sample solution and 33 µl of the aqueous solution of mushroom tyrosinase (1380 units/ml) were added to the mixture. The initial rate of linear increase in optical density at 470 nm, based on the formation of dopachrome, was measured immediately. All the extracts were first dissolved in dimethyl sulfoxide (DMSO) and used for the actual experiment at 30 times dilution. The final sample concentration for the test was 100 µg/ml. DMSO, without test extracts, was used as control. Kojic acid was used as a positive control. The relative tyrosinase activity was calculated according to Eq. 3:

$$\text{Relative tyrosinase activity}\ (\%) = \left(\frac{\text{Act}_s}{\text{Act}_c}\right) \times 100 \quad (3)$$

where Act_s is the activity of the sample and Act_c is the activity of the control.

Antifungal assay

The antifungal assay was conducted using homogenized hyphae of *Trametes versicolor* according to the method described by Kawamura et al.[14] *Trametes versicolor* FFPRI 1030 was incubated for 5 days in liquid medium prepared by dissolving 4.0 g of malt extract, 0.2 g of peptone, and 0.4 g of glucose in 200 ml of distilled water. After the incubation the hyphae were homogenized, the liquid medium was removed by centrifugation, and the homogenized hyphae were washed with physiological saline. The obtained homogenized hyphae were added to 12 ml of 3.9% sterilized potato dextrose agar (PDA) medium (0.47 g of PDA dissolved in 12 ml of water), and mixed thoroughly. The medium containing hyphae was then poured into a 9-cm petri dish, and placed in a laminar flow cabinet until the medium had solidified. Sterilized paper disks of 6 mm in diameter were permeated with 5, 10, or 20 µl of the methanol solutions containing the test methanol extracts (2, 10 µg/µl), and put on the medium. The amounts of extracts in each paper disk were equivalent to 10, 20, 40, 50, 100, and 200 µg, respectively. Paper disks permeated with hinokitiol and the solvent only were used as positive and negative controls, respectively. Incubation was conducted in covered petri dishes for 3 days at 26°C and the minimum amounts (in micrograms) required for inhibition of fungal growth were determined.

Results and discussion

The yields of the methanol extracts from the heartwoods of 22 African tropical hardwood species are listed in Table 1. The content of total phenolic compounds in these extracts, and their antioxidant activity, relative tyrosinase activity, and antifungal activity are also given in Table 1.

Extracts yields and content of total phenolic compounds

The amounts of extractives contained in the heartwoods of the 22 tropical hardwood species varied significantly. The yields of extracts ranged between 10% and 18% for 6 species, between 5% and 10% for 8 species, and less than 5% for the remaining 8 species. The content of total phenolic compounds in the extracts also differed considerably, and ranged from more than 800 mg/g to less than 100 mg/g. It was noted that the heartwoods of *Cylicodiscus gabunensis* (denya) and *Terminalia ivorensis* (emire) contained large amounts of phenolic compounds. The yields of methanol extracts of these two species were more than 15%, and the phenolic compounds constituted more than 60% of the total extracts, 868 mg/g for denya and 607 mg/g for emire. In contrast, the content of total phenolic compounds in the methanol extracts of *Guarea cedrata* (guarea) and *Nauclea diderrichii* (kusia) were very low, only 93 mg/g and 71 mg/g, respectively.

Antioxidant activity

The IC_{50} values of the methanol extracts (Table 1) indicate that the antioxidant activity of the 22 methanol extracts differed significantly. The extracts from 13 species had considerably high antioxidant activity, with IC_{50} values below 10 μg/ml. The extracts from denya and emire showed very high antioxidant activity. The IC_{50} values of denya and emire were 1.4 μg/ml and 2.5 μg/ml, respectively. The IC_{50} value of (+)-catechin, a very common natural antioxidant and used as positive control in this study, was 2.1 μg/ml. This result indicates that the crude methanol extracts from denya exhibited stronger antioxidant activity than (+)-catechin, and the extracts from emire had similar antioxidant potential to (+)-catechin.

The relationship between the relative antioxidant activity and the content of total phenolic compounds in the extracts is given in Fig. 1. The results show that, in general, the antioxidant activity increases with the content of total phenolic compounds in the extracts, and that there is a good correlation between the relative antioxidant activity and the content of total phenolic compounds. Therefore, it can be concluded that the phenolic compounds in the extracts were the major components contributing to the antioxidant property of the heartwood extracts. This has also been observed by other researchers.[15,16]

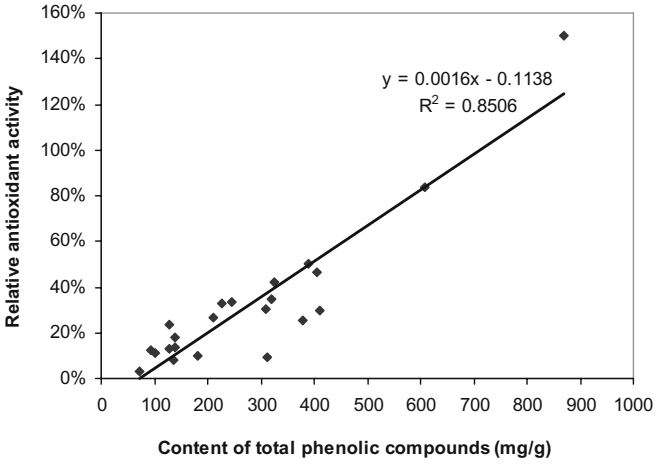

Fig. 1. Correlation between the relative antioxidant activity of the methanol extracts from the heartwoods of 22 African tropical hardwood species and the content of total phenolic compounds in the extracts [expressed as (+)-catechin equivalent]

Tyrosinase inhibitory activity

Among all 22 species, only 1 species, *Milicia excelsa* (odum), contained extractives that exhibited very high tyrosinase inhibitory activity. The extracts from other species showed very weak or no tyrosinase inhibitory property (see Table 1). Odum has been observed to have high tyrosinase inhibitory activity and chlorophorin was elucidated as a major component contributing to the tyrosinase inhibitory activity in our previous report.[17] This is confirmed in the current study.

Antifungal activity

The minimum amount of extracts for inhibiting the growth of *Trametes versicolor* hyphae and forming an inhibitory circle around the paper disks on the PDA medium was used to evaluate the antifungal properties. The extracts with a minimum inhibitory amount of 50 μg or less were classed as having high antifungal activity. As shown in Table 1, the methanol extracts from nine species had high antifungal activity. We compared the minimum inhibitory amounts with the contents of the total phenolic compounds in the extracts as well as their relative antioxidant activity. It can be concluded that there is no correlation between antifungal property and the content of total phenolic compounds in extracts and the antioxidant activity. Although some researchers have assumed that antioxidant activity may be a factor contributing to the antifungal activity,[18] our study shows no direct relationship between antifungal activity and antioxidant activity.

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