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NOTE

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Antimicrobial activity against *Streptococcus sobrinus* and glucosyltransferase inhibitory activity of taxifolin and some flavanonol rhamnosides from kempas (*Koompassia malaccensis*) extracts

Received: September 22, 2008 / Accepted: January 19, 2009

Abstract Twenty plant materials collected from the islands of Java and Kalimantan in Indonesia were extracted with 50% aqueous ethanol (crude extract). The crude extracts were assayed for antimicrobial activities against *Streptococcus sobrinus* and for glucosyltransferase (GTase) inhibition. Fourteen extracts inhibited the growth of *S. sobrinus* by more than 50% and six extracts inhibited GTase activity by more than 50% at a concentration of 100 µg/ml. *Koompassia malaccensis* (kempas) extracts showed 90% depression of *S. sobrinus* growth and 80% inhibition of GTase activity at a concentration of 100 µg/ml. Kempas crude extracts were subjected to column chromatography using Sephadex LH-20 and then preparative high-performance liquid chromatography to isolate four compounds A, B, C, and D. These compounds were identified as taxifolin and the flavanonol rhamnoside isomers neoastilbin, astilbin, and isoastilbin, respectively, from ¹H and ¹³C nuclear magnetic resonance (NMR) spectra and other two-dimensional NMR techniques (COSY, HMBC, and HMQC). Each compound depressed the growth of *S. sobrinus* over a concentration range of 9.3–42.7 µg/ml and showed GTase inhibitory activity with IC₅₀ values in the range 27.4–57.3 µg/ml. Taxifolin and flavanonol rhamnoside isomers isolated for the first time from kempas could be potent compounds for preventing dental caries.

Key words Kempas · *Streptococcus sobrinus* · Taxifolin · Neoastilbin · Astilbin

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Part of this report was presented at the Japan Wood Research Society Conference, Hiroshima, 2007

Introduction

Dental caries is a transmissible infectious disease and one of the most common infectious diseases afflicting humans.¹ Mutans streptococci (*Streptococcus mutans* and *Streptococcus sobrinus*) are considered to be major dental caries agents. They are the most common pathogens isolated from human dental plaque and their prevalence has been reported in epidemiological studies.² Several epidemiological studies have shown that the prevalence of *S. sobrinus* is more closely associated with high caries activities.³ Mutans streptococci grow inside the oral cavity and convert sucrose to insoluble adhesive glucans, which, in turn, attach to the surface of the teeth while oral bacteria produce organic acids that break down the enamel of the tooth surface. This process is recognized as the primary stage of cavity development.

Different strategies for preventing dental caries caused by cariogenic bacteria, such as depression of the growth of *Streptococcus*, inhibition of glucosyltransferase (GTase) activity, and hydrolysis of glucans by enzymes, have been developed. Although control of cariogenic bacteria by antibiotics seems to be an effective temporary treatment, it cannot be used over a long term because of the onset of antibiotic-resistant bacteria. Therefore, studies to find alternatives to antibiotics from medicinal plants are desirable. Many demonstrations on antimicrobials and GTase inhibitors have been attempted for various materials such as herbs, fruits, and barks. Medicinal plants have been used in traditional folk medicines for thousands of years, and have shown promise as sources of components for developing new antibacterial, antifungal, antiviral, anticancer, and anti-hypertensive drugs.⁴

In the dental field, several studies have also shown the feasibility of using medicinal plants as preventive means against oral diseases, particularly dental plaque-related diseases such as dental caries.⁵ Components with antimicrobial and GTase inhibitory properties against dental pathogens have been derived from plants, food, and common beverages, such as black tea, green tea, oolong tea,⁶ bark

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proanthocyanidins,⁷ cocoa bean husk extract,⁸ propolis,⁹ apple polyphenols,¹⁰ water-soluble extract of cacao,¹¹ high molecular weight components of cranberry,¹² and *Dryobalanops* sp. heartwood extracts.¹³

Thousands of other potentially useful plants have not been tested. The abundant natural resources of Indonesia have been used for foods and medicines by many Indonesian tribes with years of experience and empirical knowledge. According to historical findings, several woods and plants species from Indonesia have been collected and investigated for antimicrobial action to *S. sobrinus* and GTase inhibition.

Among the samples, this study investigated the active constituents of kempas. Kempas is an important, valuable wood because it is used as flooring, moldings, furniture, and veneer in Indonesia. Kempas belongs to the Leguminosae family and is widely distributed in Sumatra and Kalimantan. The bark is traditionally used to prepare medicinal baths because of its antifever and antidyentery activities.¹⁴ Many plants in Leguminosae show bioactivities such as antidiabetic, antimalaria, and antioxidant activity but little is known about the antimicrobial activity against oral pathogens or the effects on dental plaque formation in vitro. Therefore, the aim of this study was to isolate and identify the active compounds that depress the growth of cariogenic bacteria and inhibit GTase activity. Taxifolin and some flavanone rhamnosides were isolated and identified from kempas extracts that showed depression of growth of *S. sobrinus* and inhibition of GTase.

Materials and methods

Plant materials

Twenty plant materials were collected from Indonesia. Ten herbal plants were obtained from the islands of Java and another ten woody plants were provided by the Department of Forest Product Technology, Mulawarman University, Kalimantan Island. These plants were used in the screening test for antimicrobial action against *Streptococcus sobrinus* and GTase inhibition. The voucher specimen of *Koompassia malaccensis* (FHT.LA.13.11m) was deposited at the Wood Anatomy Laboratory of Mulawarman University, Indonesia.

Preparation of plants extracts

The dried samples (50 g) were extracted with 250 ml of 50% ethanol. After evaporating the extracts to dryness, the extracts were dissolved in 40% ethanol to measure the antimicrobial activity to *S. sobrinus* and GTase inhibitory activity. The 50% ethanol extracts were regarded as crude extracts in this study. The crude extracts were subjected to screening for antimicrobial and GTase inhibitory activity. The most active crude extracts in the assays were further fractionated.

Antimicrobial activity assay

The microorganism used in this study was *Streptococcus sobrinus* 6715, which is a proven cariogenic pathogen. Because mutans streptococci is involved in caries formation, we choose *S. sobrinus* as the test microorganism in this study. *Streptococcus sobrinus* 6715 was cultured on Todd Hewitt broth.¹⁵ The crude extracts were tested for antibacterial activity in sterile 96-well plates. Fifty microliters of microbial inoculum was added to each well containing sample and medium to achieve a final volume of 200 μ l and a final sample concentration of up to 225 μ g/ml. The test extract was prepared in a concentration range of 3.5–225 μ g/ml using a two-fold dilution method. Solvent and medium controls were included on each test plate. In order to dissolve the sample extracts, 40% ethanol was used in this study, which showed no significant inhibitory effect on *S. sobrinus* growth. The final concentration of ethanol in the well was less than 8% (preliminary analyses with 8% v/v), and for each experiment there was a growth control with and without ethanol. Furthermore, the bacterial inhibitory effects of isolated compounds were also measured. Triplicate samples were taken for each test concentration. After incubation for 12 h at 37°C, *S. sobrinus* growth was estimated spectrophotometrically at 590 nm using a microplate reader. Percentage growth was calculated with the concentration tested to determine a concentration that inhibits 50% growth (IC₅₀).

Minimum inhibitory concentration (MIC) values were measured with a modified microdilution broth method described by Cai and Wu.¹⁶ The MIC was defined as the minimum concentration of test sample that inhibits the visible growth of microorganisms after overnight incubation. To determine the minimum bactericidal concentration (MBC), an aliquot (50 μ l) of all incubated tubes with concentrations higher than the MIC was subcultured on THB (Todd Hewitt broth) agar supplemented with 1% sucrose. MBC was defined as the lowest concentration that allows no visible growth on the medium. The experiments were performed in triplicate.

Preparation of GTase

Streptococcus sobrinus 6715 was grown for 16 h at 37°C in 4 l of THB agar. After centrifugation of the culture at 5000 rpm for 10 min, the cells were collected and then extracted with 75 ml of 8 M urea at 20°C for 1 h with stirring. The crude enzyme solution containing urea was dialyzed against 10 mM potassium phosphate buffer (pH 6) until the urea was removed entirely. One milliliter of the crude enzyme solution was pipetted into a microtube and stored in a freezer at –80°C.

Assay for GTase inhibitory activity

Insoluble glucan synthesized by GTase was measured turbidimetrically. GTase was incubated in 300 μ l of 0.1 M

phosphate buffer (pH 6.0) containing 1% sucrose, 0.1% sodium azide, 0.5% dextran T-10, and in the presence or absence of a sample at 37°C for 3 h. The volume of the crude GTase solution to use in the assay was determined by that giving an absorbance of around 1.0 at 590 nm. The inhibition rate is expressed by the following equation: Inhibition rate (%) = 100 × (Ac – As)/Ac, where Ac and As are the absorbance of the control and the sample, respectively.) IC₅₀ is the sample concentration (µg/ml) giving 50% inhibition of GTase.

Fractionation of kempas extracts

Kempas heartwood meal was prepared by grinding wood chips. One hundred grams of wood meal was extracted with 3 l of 50% aqueous ethanol. The 50% aqueous ethanol extracts were evaporated to obtain 2.6 g of extract. The crude extracts of kempas were analyzed by high-performance liquid chromatography (HPLC) on a VP-ODS (250 mm × 4.6 mm i.d.) column at flow rate of 1 ml/min. The solvent system used was as follows: a gradient program for 50 min from 10% to 80% of solvent B (100% methanol) in solvent A (0.01% trifluoroacetic acid in H₂O). Elution peaks were monitored at 280 nm.

The crude extract was directly subjected to column chromatography on LH-20 stationary phase with 50% aqueous ethanol to give nine fractions (Fr. I–IX). Fractions VI–IX, which contained several predominant peaks (compounds A, B, C, and D) were then purified by preparative HPLC.

Isolation and identification of compounds A, B, C, and D

The obtained fractions were purified by preparative HPLC with reversed phase column Inertsil ODS-03 (GL Sciences, 20 mm i.d. × 250 mm) monitored at 280 nm. The solvent system used was as follows: a gradient program for 50 min from 10% to 80% of solvent B in solvent A at a flow rate of 10 ml/min. Fraction VI gave taxifolin (compound A), Fr. VII gave neoastilbin and astilbin (compounds B and C), Fr. VIII gave astilbin (compound C), and Fr. IX gave isoastilbin (compound D). The ultraviolet (UV) and nuclear magnetic resonance (NMR) spectra were recorded on a Shimadzu UV-spectrophotometer and a JEOL JNM Alpha-600 spectrometer, respectively. The compounds were identified by comparison of the spectral data with those in the literature.^{17–19}

Taxifolin. Compound A was obtained as brown powder, and exhibited an [M+H]⁺ ion peak at *m/z* 305 (M⁺ = C₁₅H₁₂O₇) in positive fast atom bombardment mass spectrometry (FAB-MS). ¹H NMR (CD₃OD, 600 MHz) δ: 4.49 (1H, d, *J* = 11.7 Hz, 3-H), 4.90 (1H, d, *J* = 11.7 Hz, 2-H), 5.86 (1H, s, 6-H), 5.90 (1H, d, *J* = 2.0 Hz, 8-H), 6.78 (1H, d, *J* = 8.2 Hz, 5'-H), 6.82 (1H, dd, *J* = 8.2, 1.4 Hz, 6'-H), 6.93 (1H, d, *J* = 2.0 Hz, 2'-H). ¹³C NMR (CD₃OD, 150 MHz) δ: 77 (3-C), 84 (2-C), 95 (8-C), 96 (6-C), 103 (10-C), 115 (2'-C), 115 (3'-C),

115 (5'-C), 120 (6'-C), 128 (1-C), 146 (4'-C), 163 (9-C), 167 (5-C), 170 (7-C), 196 (4-C).

Neoastilbin. Compound B was obtained as brown powder, and exhibited an [M+H]⁺ ion peak at *m/z* 451 (M⁺ = C₂₁H₂₂O₁₁) in positive FAB-MS. ¹H NMR (CD₃OD, 600 MHz) δ: 0.88 (3H, d, *J* = 6.2 Hz, 6''-H), 2.27 (1H, m, 5''-H), 3.17 (1H, t, *J* = 9.6 Hz, 4''-H), 3.32 (1H, dd, *J* = 3.5, 3.4 Hz, 3''-H), 3.98 (1H, m, 2''-H), 4.62 (1H, d, *J* = 11.7 Hz, 3-H), 4.96 (1H, d, *J* = 11.7 Hz, 2-H), 5.13 (1H, d, s, 1''-H), 5.86 (1H, d, *J* = 2.0 Hz, 6-H), 5.89 (1H, *J* = 2.0 Hz, 8-H), 6.78–6.82 (2H, m, 5'-H, 6'-H), 6.94 (1H, s, 2'-H). ¹³C NMR (CD₃OD, 150 MHz) δ: 17 (6''-C), 69 (5''-C), 71 (2''-C), 72 (3''-C), 76 (4''-C), 76 (3-C), 82 (2-C), 95 (6-C), 96 (8-C), 102 (10-C), 102 (1''-C), 114 (5'-C), 115 (2'-C), 120 (6'-C), 129 (1'-C), 145 (3'-C), 146 (4'-C), 164 (9-C), 167 (5-C), 177 (7-C), 195 (4-C).

Astilbin. Compound C was obtained as brown powder, and exhibited an [M+H]⁺ ion peak at *m/z* 451 (M⁺ = C₂₁H₂₂O₁₁) in positive FAB-MS. ¹H NMR (CD₃OD, 600 MHz) δ: 1.15 (3H, dd, *J* = 6.2, 5.5 Hz, 6''-H), 3.20 (1H, overlapped, 4''-H), 3.32 (1H, m, 3''-H), 3.50 (1H, m, 2''-H), 4.02 (1H, s, 1''-H), 4.22 (1H, m, 5''-H), 4.55 (1H, d, *J* = 10.3 Hz, 3-H), 5.05 (1H, d, *J* = 10.3 Hz, 2-H), 5.86 (1H, d, *J* = 2.0 Hz, 6-H), 5.88 (1H, *J* = 2.0 Hz, 8-H), 6.70–6.80 (2H, m, 5'-H, 6'-H), 6.93 (1H, *J* = 2.0 Hz, 2'-H). ¹³C NMR (CD₃OD, 150 MHz) δ: 17 (6''-C), 71 (5''-C), 72 (2''-C), 72 (3''-C), 74 (4''-C), 77 (3-C), 96 (2-C), 96 (6-C), 96 (8-C), 101 (1''-C), 103 (10-C), 116 (5'-C), 119 (2'-C), 122 (6'-C), 131 (1'-C), 147 (3'-C), 147 (4'-C), 164 (9-C), 168 (5-C), 168 (7-C), 197 (4-C).

Isoastilbin. Compound D was obtained as brown powder, and exhibited an [M+H]⁺ ion peak at *m/z* 451 (M⁺ = C₂₁H₂₂O₁₁) in positive FAB-MS. ¹H NMR (CD₃OD, 600 MHz) δ: 0.90 (3H, d, *J* = 6.18 Hz, 6''-H), 2.45 (1H, m, 5''-H), 3.57 (1H, m, 4''-H), 3.65 (1H, m, 3''-H), 4.16 (1H, d, *J* = 2.8 Hz, 2''-H), 4.60 (1H, d, *J* = 2.1 Hz, 3-H), 5.20 (1H, d, *J* = 2.1 Hz, 2-H), 5.40 (1H, d, *J* = 2.0 Hz, 1''-H), 5.95 (1H, d, *J* = 2.1 Hz, 6-H), 6.78 (1H, d, *J* = 2.1 Hz, 8-H), 6.80 (2H, dd, *J* = 8.2, 2.0 Hz, 5'-H, 6'-H), 6.92 (1H, d, *J* = 1.2 Hz, 2'-H). ¹³C NMR (CD₃OD, 150 MHz) δ: 17 (6''-C), 69 (2''-C), 71 (3''-C), 74 (3-C), 74 (4''-C), 74 (5''-C), 81 (2-C), 81 (1''-C), 99 (6-C), 99 (8-C), 105 (10-C), 115 (5'-C), 115 (6'-C), 118 (2'-C), 128 (1'-C), 147 (3'-C), 149 (4'-C), 164 (9-C), 165 (5-C), 166 (7-C), 195 (4-C).

Results and discussion

In this study, 50% ethanol extracts of 20 materials from Indonesian plants were evaluated for their antibacterial activities and GTase inhibitory activities. In the screening experiments, most of the samples had activities to depress *Streptococcus sobrinus* bacterial growth. *Curcuma xanthorrhiza* (temulawak) and *Koompassia malaccensis* (kempas) especially showed strong growth inhibitory activity as shown in Table 1.

Table 1. Inhibitory activities of 50% ethanol extracts of 20 Indonesian plants against *Streptococcus sobrinus* growth and glucosyltransferase (GTase) activity

No	Sample name	Scientific name	Family name	Part	Yield ^a (%)	Inhibitory activity ^b (%)	
						<i>S. sobrinus</i>	GTase
1	Mahoni	<i>Swietenia macrophylla</i>	Meliaceae	Wood	4.5	65 ± 2.0	91 ± 1.5
2	Avicenia	<i>Avicenia officinalis</i>	Acanthaceae	Wood	3.2	71 ± 0.4	–
3	Waru	<i>Hibiscus tilliaceus</i>	Malvaceae	Wood	5.3	65 ± 7.8	9 ± 12.3
4	Medang	<i>Intsia palembanica</i>	Leguminosae	Wood	4.3	54 ± 0.9	34 ± 5.6
5	Rhizopora	<i>Rhizopora</i> sp.	Rhizoporaceae	Wood	12.3	44 ± 5.3	91 ± 7.7
6	Boli	<i>Xylocarpus granatum</i>	Meliaceae	Wood	6.5	33 ± 12.8	91 ± 5.3
7	Kempas	<i>Koompasia malaccensis</i>	Leguminosae	Wood	2.2	91 ± 3.5	81 ± 2.4
8	Merbau	<i>Litsea</i> spp.	Leguminosae	Wood	10.2	56 ± 9.8	58 ± 4.5
9	Palele	<i>Castanopsis javanica</i> A.DC	Fagaceae	Wood	3.4	60 ± 6.3	39 ± 1.4
10	Gelam	<i>Melaleuca leucadendron</i>	Myrtaceae	Wood	1.5	65 ± 3.6	93 ± 1.9
11	Temulawak	<i>Curcuma xanthorrhiza</i>	Zingiberaceae	Rhizome	15.5	96 ± 1.0	23 ± 2.2
12	Mahkota dewa	<i>Phaleria papuana</i>	Thymelaeaceae	Leaf	9.5	65 ± 5.8	13 ± 4.1
13	Mahkota dewa	<i>Phaleria papuana</i>	Thymelaeaceae	Fruit	20.3	22 ± 1.9	12 ± 0.9
14	Sambiloto	<i>Andrographis paniculata</i>	Acanthaceae	Herb	21.5	44 ± 0.9	15 ± 1.1
15	Daun dewa	<i>Gynura divaricata</i>	Asteraceae	Rhizome	4.8	60 ± 2.4	28 ± 1.5
16	Brotowali	<i>Tinospora crispa</i> (L) Miers	Menispermaceae	Stem	21.0	63 ± 1.7	3 ± 3.7
17	Daun dewa	<i>Gynura divaricata</i>	Asteraceae	Leaf	3.9	44 ± 7.3	–
18	Jambu	<i>Psidium guava</i>	Myrtaceae	Leaf	13.6	69 ± 2.9	89 ± 5.7
19	Jati Belanda	<i>Guazuma ulmifolia</i>	Malvaceae	Leaf	11.7	39 ± 5.5	–
20	Sirih merah	<i>Piper crocatum</i>	Piperaceae	Leaf	10.7	54 ± 11.5	22 ± 7.9

Data given as mean ± standard deviation, $n = 3$

^aBased on dry materials

^bInhibitory activities were assayed at the concentration of 100 µg/ml

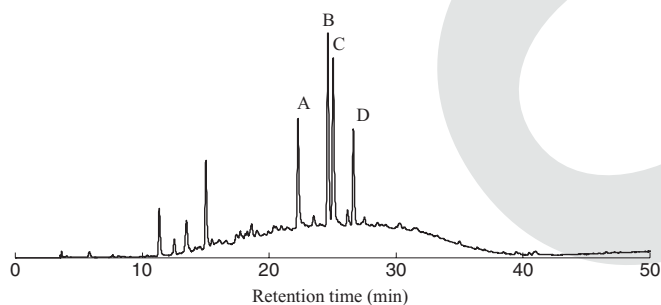


Fig. 1. High-performance liquid chromatography chromatogram of kempas crude extract. A, Taxifolin; B, neoastilbin; C, astilbin; D, isoastilbin. Conditions: column, VP-ODS (250 × 4.6 mm i.d.); gradient program, methanol/0.05% trifluoroacetic acid = 10/90 to 80/20 (40 min), 100/0 (50 min); detection wavelength, 280 nm; flow rate, 1 ml/min; analysis time, 50 min

Hwang and coworkers²⁰ reported that the extracts of temulawak showed antibacterial activity against *S. sobrinus* and *Streptococcus mutans*; in particular, xanthorrhizol strongly inhibited the growth of *S. sobrinus* with a MIC of 4 µg/ml. On the other hand, there have been no reports of kempas extracts related to antibacterial activity. Therefore, we investigated the isolation and identification of antibacterial active compounds contained in kempas against *S. sobrinus*.

Figure 1 shows the HPLC chromatogram of crude extract from kempas. The extract was purified by preparative HPLC to isolate four compounds, which were identified by NMR spectroscopy. The UV-visible spectra of major component peaks A, B, C, and D were similar to that of taxifolin. By analysis of the NMR data that was generated in this

study, and by comparison of the physical and spectral data with those reported in the literature, compound A was identified as taxifolin.²¹

The ¹³C NMR spectrum of compound A exhibited signals typical of the flavonol-type skeleton: 83 ppm at C-2 and 77 ppm at C-3. The saturated bond between C-2 and C-3 was confirmed by the presence of doublets at 4.90 ppm (H-2) and 4.49 ppm (H-3) in the ¹H NMR spectrum. Thus, compound A was identified as dihydroquercetin, otherwise known as taxifolin. The flavonol-like taxifolin signals were also observed in the ¹H NMR spectra of compounds B, C, and D. In addition, glycoside signals were observed at 4.02–5.40 ppm, 3.50–4.16 ppm, 3.32–3.65 ppm, 3.17–3.57 ppm, 2.27–4.22 ppm, and 0.88–1.15 ppm, which indicates the presence of a rhamnose residue. Furthermore, two-dimensional heteronuclear multiple bond coherence (HMBC) NMR spectroscopy of these compounds showed a correlation between the anomeric proton of rhamnose and the C-3 carbon of the flavanone, which indicates that rhamnose should be connected with C-3 of the taxifolin moiety (Fig. 2). Therefore, compounds B, C, and D are assumed to be variants of the flavanone-3-O-rhamnoside structure. After comparing ¹H NMR data from the literature, compounds B, C, and D were identified as neoastilbin,¹⁷ astilbin,¹⁸ and isoastilbin,¹⁹ respectively, as illustrated in Fig. 2.

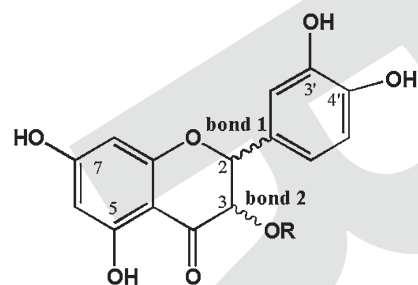
Streptococcus sobrinus growth inhibitory activities were investigated for compounds A, B, C, and D. Their activities increased in accordance with concentration (data not shown). MIC, MBC, and IC₅₀ for *S. sobrinus* growth inhibitory activities are shown in Table 2. The crude extract and each isolated compound had MIC values of 225 µg/ml. These MBC values were higher than the MIC values except for the crude extract. Each compound depressed the growth

Table 2. Antimicrobial and GTase inhibitory activities of 50% ethanol extracts and compounds isolated from kempas

No.	Sample	Antimicrobial activity			IC ₅₀ against GTase (µg/ml)
		MIC (µg/ml)	MBC (µg/ml)	IC ₅₀ (µg/ml)	
1	Kempas crude extract	225	900	9.3 ± 1.7	27.4 ± 0.6
2	Taxifolin	225	900	21.8 ± 1.7	53.0 ± 0.7
3	Neoastilbin	225	450	16.5 ± 1.5	5.0 ± 1.3
4	Astilbin	225	900	22.8 ± 1.9	57.3 ± 0.7
5	Isoastilbin	225	900	42.7 ± 1.5	54.3 ± 0.9

Data given as mean ± standard deviation, $n = 3$

MIC, Minimum inhibitory concentration; MBC, minimum bactericidal concentration; IC₅₀, concentration that inhibits 50% of targeted activity



Compound	Bond 1	Bond 2	R	Name
A	-----	▀	H	Taxifolin
B	▀	-----	Rhamnoside	Neoastilbin
C	-----	▀	Rhamnoside	Astilbin
D	-----	-----	Rhamnoside	Isoastilbin

Fig. 2. Structures of taxifolin and flavanone rhamnosides isolated from 50% ethanol extracts of *Koompassia malaccensis* wood

of *S. sobrinus* over a concentration range of 9.3–42.7 µg/ml. The antibacterial activity of taxifolin and flavanone rhamnosides from kempas may be due to their chemical structure. Many studies have reported relationships between antimicrobial activity and the chemical structure of flavonoids. Indeed the presence of 3',4'-hydroxyl units is essential for the activity of flavanones.²² In addition, dihydroxylation at the 5-position and 7-position is essential for the antimicrobial activity of flavanones because 5,7-dihydroxyl groups are commonly present in all active flavanones but not in inactive ones.²³ There may have been more active compounds than those isolated compounds in the crude extracts, but we were unable to isolate the potent active compound in this experiment.

These compounds also inhibited glucosyltransferase secreted from *S. sobrinus* to a remarkable extent with 60%–80% inhibition (data not shown), and the IC₅₀ values of the crude extract, compounds A, B, C, and D were 27.4, 53.0, 5.0, 57.3, and 54.3 µg/ml, respectively (Table 2). Judging from the IC₅₀ values, compound B (neoastilbin) shows remarkable inhibition of GTase activity with 5.0 µg/ml. Oligomeric proanthocyanidins, so-called condensed tannins, are contained in bark, oolong tea, green tea, and grape seed and are potent GTase inhibitors owing to their polyphenolic moiety being able to form hydrogen bonds with GTase. However, neoastilbin showed quite potent GTase inhibi-

tory activity in spite of being a low molecular weight polyphenolic compound, which might indicate that neoastilbin inhibits GTase with an inhibition mode unlike the proanthocyanidins.

Conclusions

The antimicrobial activity against *Streptococcus sobrinus* and the inhibitory activity against GTase were assayed for 50% ethanol extracts from 20 Indonesian plants. The wood extract of *Koompassia malaccensis* (kempas) was chosen for further investigation of the isolation procedure and the identification of bioactive compounds because of its good activities in both assays. Taxifolin and three flavanone rhamnoside isomers, neoastilbin, astilbin, and isoastilbin, were isolated and identified as bioactive compounds. Among these isolated compounds, neoastilbin showed an especially potent GTase inhibitory activity. The strong inhibitory activity against GTase is very interesting, because neoastilbin is a relatively smaller compound than oligomeric polyphenols, which are considered to be strong inhibitors. The stereochemistry at C2 and C3 of taxifolin largely governs the potency of inhibition, and controls the steric configuration of the rhamnose moiety. Because of its promising display of antimicrobial and GTase inhibitory activity shown here, taxifolin and flavanone rhamnosides can also be considered for further pharmacological studies related to the development of a natural anticariogenic agent for dental caries. Although the isolated compounds in this study were not the most active compounds, they may represent another source of active compounds from kempas crude extracts with stronger activity. The isolation and identification of others compounds from kempas should be examined in the future.

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